Neurocognitive impairments and neuropsychiatric risk in 22q11 Deletion Syndrome.

Kate Deborah Baker

September 2003

Behavioural and Brain Sciences Unit
Institute of Child Health
University College London

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

This thesis is dedicated first and foremost to all of the young people who participated in the studies presented. They have inspired and puzzled me in equal measure. Secondly, to their parents, and to my own parents, who have taught me more about courage and caring than they perhaps realise.

Abstract

22q11 Deletion Syndrome (22q11DS) is associated with somatic anomalies, learning disability, and psychiatric disturbance; a high rate of schizophrenia (10-40%) has been reported in affected adults. The aim of this study was to acquire evidence for genetic disruption of specific developmental processes that may explain the later emergence of psychotic illness in 22q11DS.

Neurobiological traits associated with risk for psychosis (endophenotypes) were selected for investigation following review of the existing literature on genetics, neurodevelopment and schizophrenia, and were investigated using cognitive and EEG event-related potential (ERP) methodologies. Adolescents and young adults with 22q11DS were compared to age- and IQ-matched controls on all measures.

Between-groups analysis revealed abnormalities of auditory processing, working memory and expressive language in 22q11DS. ERPs provided strong evidence for schizophrenia-like disruption; mismatch negativity (MMN), an ERP elicited by any discriminable change in a repetitive auditory sequence, was reduced in amplitude at frontal electrodes but not at temporal sites. Anomalous context-dependence of speech MMN was also observed; individuals with 22q11DS displayed a specific deficit in eliciting MMN in response to voicing contrasts between speech sounds. No such deficits were found in children with Specific Language Impairment, although other abnormalities in auditory processing were apparent.

Within-group analysis indicated that abnormal auditory ERPs were associated with psychiatric symptoms akin to schizotypal personality disorder in some 22q11DS individuals. The association between these impairments supports the view that MMN indexes neurophysiological processes relevant to psychotic illness. The severity of neurocognitive abnormalities in 22q11DS was found to be modulated by the *catechol-o-methyl transferase* $^{met}158^{val}$ polymorphism on the single non-deleted chromosome 22. Functional variants of the *COMT* gene have been associated with risk for schizophrenia and schizophrenia-like cognitive abnormalities in the general population. Thus developmental dysregulation of catecholamine systems may at least partially explain the association between 22q11DS and psychosis.

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Acknowledgments

The involvement of 22q11DS individuals and their families in this study would not have been possible without the assistance of the UK 22q11DS Group, and Linda Campbell at the Institute of Psychiatry.

South Camden Community School (Camden), Cardinal Pole School (Hackney), Bishop Ramsey School (Hillingdon), Mellow Lane School (Hillingdon), Loxford School (Redbridge), Richmond College of Further Education, the Leighton Project (Camden), CityLit and KEEN Oxford assisted in the recruitment of control participants.

The opportunity to investigate a group of children with Specific Language Impairment as part of this study was offered by Professors Faraneh Vargha-Khadem and Susan Gathercole, and was carried out with the assistance of Drs Peter Rankin and Josie Briscoe.

All genetic analysis (presented in Chapter 8) was carried out by Dr Sinthuja Siva under the supervision of Professor Pete Scambler, in the Molecular Medicine Unit at the Institute of Child Health.

Neurophysiological experiments were carried out in the Clinical Neurophysiology department of Great Ormond Street Hospital. Dr Stewart Boyd and staff provided space, equipment, time and technical assistance.

Amita Jassi and Lorna Nelson provided essential administrative support.

Dr Torsten Baldeweg was an invaluable source of expertise and ideas, and provided training, supervision, and many enlightening discusssions.

Professor David Skuse supervised this investigation with patience and enthusiasm.

This study was funded by PPP Healthcare Research and by the Child Health Research Action Trust.

Andy Hull was Editor-in-Chief.

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1 Introduction

1.1 Overview

In this investigation, a small group of adolescents and young adults representing a rare population (22q11DS) has been studied, because of the population-based association with unusually high rates of schizophrenia. Our present understanding of the syndrome and its relationship to psychosis is extremely limited. No gene has been implicated as the causal factor for psychiatric illness or cognitive disability in 22q11DS, and no neural or developmental mechanism has been provided to explain the association with schizophrenia. 22q11DS is unique as a high-risk population because of its aetiological dependence on a specific genetic anomaly. As the only known developmental syndrome associated with a schizophrenia-like psychopathological phenotype, 22q11DS is an unparalleled model population in whom to study gene-brain-behaviour relationships.

In this introductory section, 22q11DS and its association with psychosis will be described. Evidence for genetic and neurodevelopmental influences in schizophrenia will be discussed. The principles of identifying genetic influences on mental health via the elucidation of endophenotypes will be introduced and discussed, with reference to contemporary examples in schizophrenia research. Lastly, the aims of the current study will be stated and discussed.

1.2 22q11 Deletion Syndrome

1.2.1 Introducing 22q11DS

22q11 Deletion Syndrome (22q11DS) is a collective genetic diagnosis referring to a group of congenital anomaly syndromes that are caused by the deletion of a portion of the long arm of chromosome 22. The microdeletion at 22q11 is found in the majority of cases of DiGeorge Syndrome (of which the main symptoms are athymaplasia, cardiac malformations and facial anomalies), VeloCardioFacial Syndrome (a broader dysmorphological description emphasising palate malformations) and other less frequent congenital anomaly syndromes (Scambler, 2000). There is much overlap between the features found in association with each of these clinical diagnoses, hence

the genetic label is increasingly favoured. Around 180 anomalies are associated with 22q11DS, and, according to currently available data, there is no single characteristic shared by all individuals with 22q11DS. The most common physical features are congenital heart defects, palate anomalies and atypical facial features, but anomalies of renal, endocrinological and immunological systems are also frequent (Ryan et al., 1997).

The majority of individuals with 22q11DS harbour a deletion of approximately 3Mb in size (the typically deleted region or TDR) that encompasses the sequence of around 40 genes. Virtually identical low-copy repeat elements are present at both the proximal and distal breakpoints of the TDR, indicating that homologous recombination during meiosis is a likely mechanism of deletion. Smaller deletions have also been reported, not all of which are overlapping, indicating that there may be multiple genes in the region contributing to developmental disorder or that chromosomal disruption may exert long-range influence on the key gene(s). The microdeletion occurs de-novo in 90 – 95% of cases and thereafter is inherited in a dominant fashion. Outcomes within families are highly variable, even in monozygotic twins (Vincent et al., 1999), indicating the large extent to which additional factors, both genetic and environmental, interact with chromosomal disruption predisposing to developmental disorder.

The spectrum of atypical physical development in 22q11DS is wide both in terms of number of features and severity of disruption in each affected individual. There are no obvious genetic causes of heterogeneity such as deletion size (Carlson et al., 1997), and there is no strong evidence for the clustering of one feature with another. For example individuals with a severe heart defect seem not to be at higher risk for learning disability or palate anomalies. This suggests that the 22q11 region includes one or more genes that independently influence the development of several physiological systems, acting in concert with the many other genes and environmental factors that influence the development of each system. Disruptions within each system when they occur tend to follow predictable patterns. For example, if a heart defect occurs it is likely to affect the integrity of the outflow tracts of the heart, giving rise to anomalies along a spectrum of severity from mild to life-limiting. This suggests that specific developmental mechanisms are regulated by molecular interactions that are dependent on two functioning copies of a gene in the 22q11 region. Haploinsufficiency of this gene or genes renders these developmental processes vulnerable to disruption, thus the

microdeletion gives rise to increased risk of many different developmental disorders that are to some extent reliant on common mechanisms.

22q11DS can also include marked neurocognitive and neuropsychiatric features. In common with the physical aspects of the syndrome, the range of severity of cognitive disability and psychiatric illness in 22q11DS is wide, and yet appears to follow common patterns. This suggests that a gene or genes in the deleted region of chromosome 22 influences brain development by specific mechanisms and that haploinsufficiency of the gene(s) renders certain developmental processes vulnerable to disruption with potentially significant but non-fatal consequences.

1.2.2 22q11DS and neuropsychiatric risk

In common with other developmental disorders which include a degree of learning disability, children and adults with 22q11DS can experience a wide range of types and severity of emotional and behavioural difficulty. An unusual feature however, and the primary motivating factor in recent research, is the reported association between 22q11 DS and schizophrenia in adulthood, which has been found to affect up to 30% of adults with the microdeletion (Table 1.1). Complementary studies indicate an elevated rate (relative to the general population rate of 0.01 - 0.025%) of the microdeletion in patients with schizophrenia who had not previously been diagnosed with a developmental syndrome (Table 1.2). A 30% lifetime rate of psychotic illness, as reported by Murphy and colleagues (1999) is 30 times higher than for the general population and about 10 times higher than that reported for mild learning disability (Turner, 1989).

Not all investigators agree that 22q11DS is associated with a psychiatric outcome that conforms to the general population diagnosis of schizophrenia. Vogels et al (2002) have instead suggested the existence of a "psychopathological phenotype" for 22q11DS on the basis of investigation of a sample of 16 VCFS patients over the age of 15 referred for neuropsychiatric evaluation. Despite the fact that all participants in this study had marked emotional and behavioural symptoms, the psychiatrists evaluating both historical and present-state information could not make a diagnosis of a standard categorical type in any case. They argue for a profile of signs and symptoms that are unique to the syndrome, which may be present to some degree in all or a subset of cases. The most prominent behavioural features are "...separation anxiety, lack of

emotional reciprocity, oppositional behaviour..., [failure to] symbolise resulting in...a paranoid attitude". They suggest that the emergence of psychotic symptoms in adolescence is an exaggeration of pre-existing features, and does not have the intrusive or frightening quality of psychosis in schizophrenia and bipolar disorder. This lack of agreement with previous reports indicates that a comprehensive examination of psychopathology in adults with 22q11DS is required, preferably with a dimensional as well as categorical approach.

Susceptibility to psychosis as a component of a behavioural phenotype in syndromes of intellectual disability is not unique, but no other syndrome currently studied has been associated with schizophrenia in the same manner as 22q11DS. Psychosis has also been reported in association with Prader-Willi syndrome (Clarke, 1998), where chromosome 15 disruption (one of several known genetic causes of this syndrome) is linked to a behavioural phenotype involving affective psychosis. This psychopathological profile has been directly contrasted to the course of psychosis in 22q11DS, which may be more chronic and less phasic, suggesting specific gene-brain-behaviour links in the two conditions (Verhoeven, Tuinier, & Curfs, 2000). Fragile X syndrome has been associated with schizotypal personality disorder in female carriers of the premutation (Sobesky, Hull, & Hagerman, 1994), but not commonly schizophrenia or other psychoses in fully affected individuals. Thus the current body of evidence suggests that individuals with 22q11DS are specifically vulnerable to mental illness that includes psychosis, and that in a higher proportion of individuals than is the case for other developmental disorders meet criteria for a diagnosis of schizophrenia.

Table 1.1 Rates of psychotic disorders in 22q11DS

Study	Sample size	Ascertainment	Age	% schizophrenia	% other diagnoses
Shprintzen et al (1992)	90	VCFS clinic	14 (mean onset of psychosis)	>10	-
Papolos et al (1996)	25	Psychiatric referral	12 (mean onset of illness)	-	64 bipolar
Murphy et al (1999)	50	Various	31 (mean)	24 (30 any psychosis)	42 any disorder
Ryan et al (1997)	252	Various	3 to 18	-	9 any disorder
Ryan et al (1997)	61	Various	18 to 51	-	18 any disorder
Arnold et al (2001)	20	Genetics clinic	6 to 20	15 displaying some schizotypal features	60 any disorder

Table 1.2 Rates of 22q11 microdeletion in other populations with psychosis

Study	Ascertainment	Population size and selection criteria [a]	Rate of detection of microdeletion
Karayiorgou et al (1995)	Psychiatric records	100 SZ or SZA	2/100 = 2%
Gauthier et al (2000)	Psychiatric records	107 SZ	3/107 = 3%
Yan et al (1998)	Drug trial	32 COSZ	1/32 = 3%
Usiskin et al (1999)	Psychiatric records	47 COSZ	3/47 = 6%
Gothelf et al (1997)	Hospital records	15 SZ with clinical features of 22q11DS	3/15 = 20%
Bassett et al (1998)	Psychiatric referrals	15 SZ or SZA with clinical features	8/15 = 53 %
Sugama et al (1999)	Psychiatric records	6 SZ with clinical features	1/6 = 17%

[a] SZ = schizophrenia, SZA = schizoaffective disorder, COSZ = childhood-onset schizophrenia

1.2.3 The search for causative genes in 22q11DS

The identification of a gene within the deleted region of chromosome 22 responsible for psychiatric disruption in 22q11DS would provide an alternative entry point for investigations into developmental and functional pathophysiology. However methods for identifying the key gene(s) in chromosomal disorders are somewhat limited. Deletion mapping - seeking a minimal region of disruption, and ideally a single gene, that is sufficient and necessary for syndromal development, of for expression of a particular aspect of the phenotype - is not possible in 22q11DS because most patients harbour large deletions with identical break-points (Carlson et al., 1997). No single-gene mutations within candidate genes in the 22q11 region giving rise to 22q11DS-like phenocopies have been identified as yet. Another strategy for identification of critical genes is to utilise animal (especially mouse) models of the genetic anomaly, to narrow down a critical region for phenotypic expression. As described below, this strategy has been successful in identifying a strong candidate gene for the cardiac phenotype in 22q11DS. However, without first identifying the causative gene for psychiatric disruption in humans, or at least defining a simple functional marker for the integrity of such a gene, it will be very difficult to conduct relevant studies of neurodevelopment in animal models, because of uncertainty as to what constitutes an appropriate mouse behavioural phenotype for the complex mental traits in question.

To date, the majority of investigations of mouse models for 22q11DS have focused on anomalies in cardiac structures and pharyngeal arch development. Large nested deletions and then targeted single gene mutations were engineered to determine a minimum region of deletion, and then a single gene, for which haploinsufficiency results in cardiac anomalies similar to those found in humans with DiGeorge Syndrome. This approach was successful in identifying *Tbx1* haploinsufficiency as a sufficient cause for the cardiac phenotype (Lindsay et al., 2001; Merscher et al., 2001). *Tbx1* is a transcription factor (regulating the expression of multiple other developmental genes) expressed during early embryological development in many tissues, including those affected in 22q11DS. As well as abnormal development of the pharyngeal arches and pouches and their derivates (heart, thymus, palate), *Tbx1* haploinsufficiency in mice is associated with misdirection of cardiac and cranial neural crest cell migration (Vitelli, Morishima, Taddei, Lindsay, & Baldini, 2002), and lack of differentiation, perhaps because of loss of soluble guidance molecules and induction factors (Kochilas et al.,

2002). *Tbx1* interacts with other pivotal factors during early cell fate specification, including *Sonic Hedgehog* (Garg et al., 2001), and members of the fibroblast growth factor (Vitelli et al., 2002) and forkhead gene families (Yamagishi et al., 2003). *Tbx1* is therefore a good candidate for a single gene cause of multi-system disruption in 22q11DS, including the syndrome's neurodevelopmental features.

Several observations question whether *Tbx1* haploinsufficiency is sufficient to account for the full spectrum of phenotypic features in 22q11DS. Patients who appear to have a full 22q11DS phenotype have been identified who harbour non-overlapping small deletions, within the larger commonly deleted region, that do not include *Tbx1* (Amati et al., 1999). This suggests that there could be several genes in a cluster at 22q11, the deletion of any of which could result in the phenotype, or that *Tbx1* could be functionally disrupted as a consequence of chromosomal distortion within a certain distance from the coding region of the gene itself. Several groups have screened patients presenting with 22q11DS-like phenotypes, but no detectable microdeletion, for mutations within *Tbx1* that would result in effective haploinsufficiency of the gene (Gong et al., 2001). No such patients have yet been found. This may indicate that *Tbx1* disruption alone could not cause the syndrome, without additive factors within the genome (either at 22q11 or elsewhere). A fascinating observation has been that the critical developmental phenotype in *Tbx1* haploinsufficient mice (abnormalities of one or both fourth pharyngeal arch arteries) is fully penetrant (present in all cases) at early stages of development (up to E10.5) but that by E11.5 these abnormalities are no longer seen in approximately one third of embryos (Lindsay & Baldini, 2001). This indicates the availability of "rescue factors", either genetic or environmental, which can counteract the effect of the genetic disruption but which are not uniformly available to every individual.

General consistency amongst mammalian species in terms of gross brain structure, patterns of regional connectivity, neurochemistry and basic molecular mechanisms of neurodevelopment suggests that modelling neurodevelopmental disruptions in mice, even those responsible for cognitive phenomena, is a valid exercise (Lipska & Weinberger, 2000). The *DF1* mouse, which harbours a deletion on murine chromosome 16 analogous to the human 22q11DS chromosomal disruption, displays a behavioural phenotype. This includes features thought to reflect schizophrenia-like neurobiological disruption, such as pre-pulse inhibition (the suppression of the startle response elicited

by a loud aversive noise, by briefly presenting the noise as a pre-pulse to the probe stimulus) and memory deficits (Paylor et al., 2001). However a behavioural phenotype has not as yet been reported for the *Tbx1* heterozygote knockout mouse, despite extensive investigation. Either *Tbx1* is not the causative gene for the behavioural phenotype and is unrelated to the cognitive and psychiatric aspects of 22q11DS, or its hemizygous deletion is not sufficient to disrupt neurodevelopment in the absence of the loss of contiguous genes. Alternatively the behavioural phenotypes tested to date may not be sensitive to *Tbx1*-dependent neurodevelopmental disruption. There are several other genes in the TDR which are good candidates for these features, including genes encoding two enzymes involved in neurotransmitter metabolism, *catechol-o-methyl transferase* (*COMT*) and *proline dehydrogenase* (*PRODH*). These genes have been implicated in schizophrenia-risk in the general population (Harrison & Owen, 2003) and mouse models mimicking disruption of both of these genes result in expression of a behavioural phenotype (Gogos et al., 1999; Huotari et al., 2002).

The key marker of 22q11DS-like cardiac phenotype in mice is absent or severely disrupted development of the fourth aortic arch. In order to determine, in mice, which gene(s) in the TDR are critical mediators of neurodevelopmental abnormality in 22q11D, a similar marker for the brain, in terms of structural anomaly, neurochemical disruption or behavioural phenotype is required. Standard tests of schizophrenia-related pathology in mice, for example pre-pulse inhibition, may not function as markers of the critical gene in 22q11DS because the neurodevelopmental pathway towards psychosis in this syndrome may be distinct from that of idiopathic schizophrenia. It is also highly plausible that the critical neurobiological substrates responsible for vulnerability to psychosis in 22q11DS are only available in the human. Without an understanding of the neurobiological substrate for abnormal mental experience in humans with 22q11DS, no animal model for the syndrome can be validly assessed with regard to a schizophrenia-relevant behavioural phenotype. The identification of candidate markers that can be translated from human to mouse is a secondary aim of the current study. Although the tests used in these human investigations do not, at present, have direct parallels in animal research, it is hoped that any positive findings could be translated into an analogous experimental paradigm for mice, be this neurobiological or behavioural.

1.3 Schizophrenia, neurodevelopment and genes

1.3.1 Introducing schizophrenia

Schizophrenia is "a severe mental disorder (or group of disorders) characterised by a disintegration of the process of thinking, of contact with reality, and of emotional responsiveness" (OCMD, 1998). Conceptual and diagnostic definitions have varied considerably in the century since formal recognition of the illness, and debate continues regarding nosological validity and utility (McKenna & van Os, 2003). Current internationally recognised criteria for diagnosing schizophrenia involve diverse symptoms - psychotic (hallucinations, delusions, thought disorder), affective, cognitive and social-occupational disruption (American Psychiatric Association, 2000). Schizophrenia, like any mental disorder, needs to be understood from many different but compatible perspectives. As noted by Littlewood (2002) the illness is "variously but convincingly described as a discrete biochemical disease with specific genetic causation, as the by-product of the evolutionary selection of creativity, as the universally recognised category of madness, as cerebral adaptation to brain damage, as faulty neurocognitive processing, as the response to faulty parenting or family communication, as the consequence of birth trauma, maternal influenza, rapid social change, capitalism, unemployment or racism, and as the social marginalization of deviance". Whilst each of these types of description can give rise to investigation within its own established and valid theoretical framework, the most exciting but difficult challenge is to map between types and levels of analysis to explore the relationships between the biological, the social and the experiential.

1.3.2 The neurodevelopmental hypothesis

Within the framework of biological psychiatry, schizophrenia is now commonly considered to be an illness of neural pathogenesis and developmental origin, caused or influenced by a larger number of genetic and other risk factors (Weinberger, 1987). Evidence for this general model (termed the neurodevelopmental hypothesis) derives from various sources, briefly summarised below. Collectively, these studies have been conceptually powerful. However progress in defining specific gene-brain-behaviour links has been slow.

1.3.2.1 Genetic evidence

The strongest known risk factor for schizophrenia is having a family history of the illness. Although absolute values for familial risk ratios vary from study to study, there is a consistent effect of genetic relatedness on degree of risk (Gottesman, 1991). The high degree to which genetic factors determine risk for schizophrenia within families has been confirmed by adoption studies; risk for the child of one affected biological parent (~13%) does not change greatly following early adoption into an unaffected family (~17%), according to data from various studies compiled by Gottesman and Shields (1982). The overall heritability (fraction of phenotypic variance in a trait that can be ascribed to additive genotypic variance within the population) can be estimated as the ratio between proportion of shared genes and proportion of shared phenotype. Heritability estimates for schizophrenia vary between 60% and 90% based on studies of either twin or family concordance rates (Tsuang, 2000).

Non-Mendelian patterns of inheritance within families, and the fact that the majority of individuals with schizophrenia do not have an affected first or even second-degree relative, implicates many different genetic pathways in the aetiology of the illness. There are likely to be multiple different genetic routes towards the illness – for some individuals one or a small number of genetic risk factors could very much increase risk whilst for others many gene variants of minor effect could combine to increase risk. Thus high heritability estimates indicating a strong genetic contribution to the illness have not in themselves assisted in the identification of specific genetic risk factors, although they have motivated molecular investigations that are beginning to yield consistent findings. In familial cases of schizophrenia with a high density of cases, many large-scale genome-wide linkage analyses have been conducted to identify chromosomal regions harbouring genes of major influence. Initial optimism about this approach, which yielded many positive results, was replaced by scepticism since there appeared to be very little consistency between studies and an enormous number of different potential loci that seemed not to generalise readily between populations. However the latest meta-analysis combining data from 20 genome-wide linkage scans by assigning ranks to loci on the basis of linkage scores for every marker tested in each scan (Lewis et al., 2003) indicates that there is more consistency than had previously been thought, with 12 loci (including a region of 22q) showing overall significant evidence for linkage to diagnosis.

Hundreds of case-control association studies have been carried out to identify potential risk alleles within polymorphic candidate genes identified as a consequence of physical linkage to a chromosomal region or on the basis of functional candidacy. This endeavour has been marred by even more inconsistencies and failures-to-replicate. There is now fairly strong evidence, however, in support of role for a number of specific genes and polymorphic markers (Harrison & Owen, 2003). More sophisticated techniques such as haplotype analysis, alongside recognition of the importance of controlling for effects of ethnic stratification and allele frequency have contributed to these successes. Further improvements, such as the ability to build polygenic models of association, plus integration of these genomic techniques with functional investigations of neurobiology and development, provide some hope for the future of this research.

1.3.2.2Neuropathological evidence

Neuropathological investigations have consistently indicated the presence of structural abnormalities in schizophrenia both at post mortem and using various in vivo imaging techniques. However, evidence for specific abnormalities in any single region of the brain has been inconsistent both between studies and between individuals within studies (Harrison, 1999). A developmental, rather than a degenerative, origin is suggested by neuropathological investigation for two reasons. Gliosis (reactive inflammatory change which would be expected if brain tissue was deteriorating prior to or during the onset of the condition) has not commonly been found, whilst cytoarchitectural abnormalities that link gross volumetric reductions to lack of neuropil volume have fairly consistently been found.

Based on 59 studies published before 1999, the only structural MRI abnormalities that are robust to meta-analysis are smaller overall brain volume (mean volume of 98% relative to controls) and increased lateral ventrical volume (mean volume 126%), neither result being functionally informative (Wright et al., 2000). Evidence for consistent reductions in the volumes of frontal and medial temporal lobe structures relative to whole brain is moderate, which may reflect that in some individuals but not others, abnormalities in these regions are predominant. The degree of individual variation in size of regional brain structures within the general population means that such small statistical differences cannot be powerful risk factors for the illness at the individual level. Newer techniques that can detect differences in the molecular

composition of the brain (magnetization transfer imaging, MTI) and in the integrity of white matter connections between brain areas (diffusion tensor imaging) have increased the ability of investigators to 'see' subtle changes and have confirmed that such changes exist in schizophrenia (Foong et al., 2001). Application of these techniques developmentally in the typically and atypically developing brain has not yet been attempted.

Recent studies by Rapoport and colleagues have indicated that divergence from normal brain development in children with early-onset schizophrenia occurs continuously, rather than existing as a fixed lesion (Thompson et al., 2001). An accelerated wave of relative grey matter loss was seen, beginning in parietal areas and later extending into the frontal lobes. A similar pattern of age-related differences was found in the well siblings of affected children suggesting genetic influence on dynamic brain change throughout development (Gogtay et al., 2003). Successive MRI scans of young adults showing psychological indicators of ultra-high risk for psychosis revealed that progressive deterioration in several brain structures occurred, in those who went on to fulfil diagnostic criteria for schizophrenia within a one year time period. These progressive changes were seen in addition to existing abnormalities that differentiated at-risk subjects from controls (Pantelis et al., 2003). Similar observations were made in the Edinburgh high risk study of adolescent offspring of schizophrenia patients in individuals who developed psychosis by their early twenties (Johnstone, Cosway, & Lawrie, 2002). An unresolved issue is whether these onset-associated brain changes are plastic, i.e. whether they return to a baseline or new state in subjects who recover well following the remission of a psychotic episode.

Collectively these studies indicate that neuroanatomical changes in schizophrenia are both developmental (and not a consequence of illness or its treatment) and progressive (through development and accelerating at the time of illness-onset). The sequence of prolonged reorganisation of the brain may show a great deal of individual variation within the general population both in terms of timing and degree of change. These progressive changes may be under partial genetic control, in that parameters influencing the process may show dependence on genetic variation. On the other hand, developmental brain changes are as likely to be influenced by environment and experience as any other aspect of age-related change. Therefore an understanding of genetic and other regulatory factors, and cognitive and psychological consequences of

dynamic brain change, would contribute to a revised and integrated model of developmental neuropathology in schizophrenia. Investigation of developmental pathways and individual variation in gene-brain-behaviour relationships in atypical groups, in particular 22q11DS, may assist in the accomplishment of this task.

1.3.2.3Epidemiological evidence

Another major strand of evidence contributing to the neurodevelopmental hypothesis is the finding from several large cohort studies that adults with schizophrenia display statistically poorer scholastic, motoric, linguistic and behavioural development throughout childhood and adolescence (Davies, Russell, Jones, & Murray, 1998). A linear relationship has been demonstrated between age of attainment of developmental milestones and likelihood of developing schizophrenia in adulthood (Isohanni et al., 2001). Both future schizophrenia cases and their well siblings investigated prospectively as part of the Philadelphia cohort born during the 1960s displayed lower performance on tests of general cognitive ability at ages 4 and 7, suggesting that these differences reflect genetically-influenced traits (Cannon et al., 2000a). This association between neurodevelopmental abnormality and schizophrenia is not found in individuals who go on to develop bipolar disorder or other psychiatric disorders, although these groups share early abnormalities in social functioning (Cannon et al., 2002).

Cohort studies focusing on later risk indicators close to the time of onset of schizophrenia have been possible due to the intensive screening protocols for military conscripts in Sweden and in Israel, with strikingly consistent results. In both studies, individuals with lower IQ and indicators of social impairment were more likely to manifest schizophrenia (but not other psychoses) within a follow-up period of several years (David, Malmberg, Brandt, Allebeck, & Lewis, 1997; Davidson et al., 1999). The Swedish conscript study (Zammit, Allebeck, Andreasson, Lundberg, & Lewis, 2002) and Dunedin cohort study (Arseneault et al., 2002) have also assessed the impact of late causal risk factors, namely cannabis use, on illness-risk whilst controlling for use of other drugs and pre-existing personality traits that may predispose to drug use. Both studies have shown that cannabis use during adolescence markedly increases risk for psychosis, although it may be impossible to separate this risk factor (potentially harmful effect of a drug) from a behavioural phenomenon linked directly with vulnerability to the illness itself. Recent studies in Holland and Denmark have returned focus to the

potential impact of the social environment as a source of risk factors. Urban life and multiple moves during childhood increase risk in a linear fashion even once other factors such as socio-economic status and family history of mental health problems are controlled for (Pedersen & Mortensen, 2001).

In summary, epidemiological studies have indicated that risk factors for schizophrenia exist from the very earliest stage of development (e.g. family history, obstetric complications) and that potential environmental hazards (e.g. cannabis use and urbanicity) continue to impact on risk during later phases of development. It is likely that mediating risk factors interact in a highly complex fashion with genetic vulnerability, and, as indicated above for cannabis use, an adverse response to a potential hazard in the environment may be as much an expression of the phenotype as an indication of causality. Non-specific neurodevelopmental abnormalities may act by reducing the threshold at which an independent neurobiological vulnerability to psychosis expresses itself, or by reducing the capacity of the individual to cope with or recover from psychiatric disturbance. Future studies will need to control for non-specific neurodevelopmental impairment to detect specific risk indicators that directly underlie psychosis. The fact that many seemingly independent risk factors act at different points in development to increase risk of developing schizophrenia has allowed the neurodevelopmental model to move beyond a "doomed from the womb" scenario of genetically-determined inevitability towards a model involving vulnerable but plastic neurobiological systems. Evidence contributing to specific features of this evolving model has so far been scarce.

1.3.3 High-risk groups: concepts of risk and evidence for neurodevelopmental disruptions

The neurodevelopmental hypothesis predicts that markers for elevated risk of schizophrenia will be present early in life, from the time at which the first causal factors act. Although the illness in its diagnostic form is latent until early adulthood, it should be associated with stable detectable traits, which are predictive of psychiatric outcome, at least at the population level. Cohort studies have indicated slight deviations in the development of motor, speech and cognitive skills, but these are highly non-specific since the vast majority of individuals displaying these features will not go on to develop schizophrenia. Identification of specific latent traits requires more intensive

investigation and longitudinal assessment of predictive power which can only be achieved in much smaller samples where the relative prevalence of the phenotype is moderately high. This can be achieved via the investigation of family members of affected individuals, most notably the offspring of patients with schizophrenia. The New York High Risk Project (Erlenmeyer-Kimling & Cornblatt, 1987), the largest and most intensive investigation of this type to date, has studied two cohorts (total 269 subjects) of children and found that as a group they were impaired, relative to control children and offspring of children of parents with affective disorders, on many different cognitive tasks and behavioural assessments both in early childhood and during adolescence. By following these children into adulthood it was possible to determine that disruptions in attention, verbal memory and motor skills during childhood (found in approximately 30% of cases for each domain) predicted adult cases of schizophrenia with a sensitivity of 57%, 84% and 75% respectively for each test domain. However the overall false positive rate (proportion of children with neurobehavioural impairment who did not go on to develop schizophrenia) was also quite high (18%, 28% and 27%) (Erlenmeyer-Kimling et al., 2000). A similar study in Edinburgh has followed a group of high-risk adolescents into young adulthood and has collected neuropsychological, psychiatric and brain imaging data. Performance on a large number of neuropsychological tests was found to be reduced in high-risk individuals, although not to the same degree as individuals with early-onset psychotic illness, and many more individuals showed evidence of neurodevelopmental impairment (both at the structural and functional level) than manifested illness (Cosway et al., 2000). These studies confirm that neurodevelopmental vulnerability is found alongside genetic risk for schizophrenia, but that making specific predictions about developmental factors associated with outcomes is a difficult, if not impossible, task.

Another strategy for defining neurocognitive abnormalities specifically associated with risk for schizophrenia is to identify adolescents and young adults who demonstrate behavioural signs of "ultra-high risk", known as prodromal features. By following such individuals for a shorter period of one or two years to determine which factors are most predictive of onset of acute psychosis, aspects of neurocognitive vulnerability immediately preceding the onset of major psychosis might be determined. McGorry and colleagues have empirically determined psychiatric criteria for inclusion into this ultra-high risk group with a positive predictive value for conversion to diagnosis within one year of about 50%, as a prelude to preventative intervention trials (Yung et al.,

2003). These findings have been very influential in transferring the focus of theoretical investigation and clinical innovation towards early identification of at-risk individuals, investigation of the mechanisms underlying progression from high-risk status to illness, and provision of interventions that aim to prevent rather than to treat psychosis. Several studies are now in progress aiming to determine the predictive power of cognitive and neurophysiological variables in young people displaying psychiatric indicators of ultra-high risk (Cadenhead, 2002).

High-risk strategies, both those involving offspring of patients and those involving clinically defined at-risk individuals, have several limitations with regard to the identification of developmental pathways and causal factors for psychosis. Firstly, heterogeneity of illness, in its symptomatic presentation, course and response to treatment, suggests that there are multiple routes to the same end-point diagnosis. Thus the power to detect consistent neurobiological anomalies may be greatly reduced. Investigation of young people with 22q11DS may be less affected by this limitation, because the common genetic aetiology underlying their high-risk status presumably acts via more homogenous pathophysiological mechanisms. Secondly, psychiatric manifestations may vary a great deal despite shared underlying neurodevelopmental disruption in terms of the type, timing and severity of symptoms. Hence many at-risk individuals defined by genetic proximity to affected individuals do not manifest the illness and are classed as "negative" cases in longitudinal analyses that aim to determine predictive factors. This is of course necessary if the aim is to identify the most at-risk individuals and to institute preventative strategies. However the factors predictive of outcome may not actually reflect the neurodevelopmental mechanisms that directly underlie vulnerability. For example, the New York High Risk Project found that poor attention was predictive of psychosis in at-risk offspring, but it may be that good attention (or verbal memory) was in fact protective against psychosis, in a manner unrelated to the underlying diathesis. By dividing the at-risk population on the basis of schizophrenia diagnosis and then working backwards to assess which neurodevelopmental factors predicted this outcome, the key neurobiological phenomenon related to illness-risk may be missed, because of low rates of conversion from at-risk state to illness.

A similar problem exists for the identification of causal genes and epigenetic factors in high-risk, family-based linkage, and case-control association studies, which are all

dependent on correct classification of individuals as "affected" or "unaffected". Family members of patients with schizophrenia display high rates of schizophrenia-spectrum conditions such as schizoaffective disorder, schizotypal personality and bipolar disorder (Kendler, Karkowski, Prescott, & Pedersen, 1998), undermining the notion that each of these disorders has a distinct genetic and neuropathological basis. (Cardno, Rijsdijk, Sham, Murray, & McGuffin, 2002) found that there was considerable shared genetic risk for bipolar disorder and schizophrenia within pairs of twins discordant for diagnosis. Relatives may share an underlying neurobiological vulnerability but experience very different symptomatology or none at all.

These limitations regarding "caseness" may apply equally to the 22q11DS group – although the rates of predicted psychotic disorder are higher in the syndrome than in familial high-risk groups, the number of individuals available for investigation is much smaller, hence the number of diagnosed cases for longitudinal analysis will be very small, and uncertainty about categorical membership is highly likely. Hence a dimensional approach for assessing the relationships between genetic risk, neurodevelopmental disruption and psychiatric risk, rather than reliance on longitudinal follow-back on the basis of categorical outcome, may be more appropriate and more informative with regard to both causes and mechanisms of symptom generation.

1.4 Looking for markers of genetically-influenced developmental disruption

1.4.1 The endophenotypes approach

The complexity of schizophrenia at each of three conceptual levels (pathological, developmental and causal) demands that in moving from general principles to specific factors and mechanisms, investigators must seek additional strategies. A change of emphasis has been advocated by some but not all investigators, away from the search for "the gene(s) for schizophrenia" and for "the brain / biochemical / cognitive basis for schizophrenia" and towards less universal but more realistic goals. These new targets could be described as "genes regulating neurobiological processes affecting individual vulnerability to components of schizophrenia". This is termed the "endophenotypes approach" (Gottesman & Gould, 2003). The aim is to identify and understand genetically-influenced neurobiological traits (endophenotypes) that are associated with

risk for developing the illness. The endophenotypes approach conceptualises vulnerability for schizophrenia in a different fashion to traditional high risk studies, by concerning itself with markers of risk-status rather than factors specifically associated with the symptomatic expression of the underlying (endophenotypic) state. An endophenotype should be a direct consequence of a disrupted developmental process, rather than a consequence of features of the illness which may result. Symptoms of the illness itself come about as the functional consequences of vulnerable neurobiological systems, as indexed by endophenotypes, in conjunction with experience and chance. Endophenotypic traits should be present in at-risk individuals whether or not they go on to manifest symptoms of the illness.

"Risk" in this context refers to population-based variance in the probability of manifesting an illness. Therefore although only 30% of individuals with 22q11DS may suffer from schizophrenia, a much higher proportion will be at risk and will display endophenotypic features. Investigation of different high-risk groups prior to the onset of a psychotic illness - 22q11DS being one such group - may assist in the task of identifying potential dimensions of neurobiological risk that influence liability to manifest the illness of schizophrenia in the general population. In addition, high risk populations, including 22q11DS, provide an opportunity to identify causal factors that may mediate or protect against both the development of the endophenotypic high-risk state and the transition in some individuals from the vulnerable state to the expression of psychiatric illness.

An endophenotype should be a continuous trait observable within the general population, the neural and molecular basis of which is tractable to investigation in large numbers of people with relative ease. Potential endophenotypes can be identified by empirical study of individuals with a diagnosis of schizophrenia or their relatives and speculation about developmental and functional cause of impairments, from retrospective study of the developmental history of sufferers, or from theoretical speculations about developmental processes which could plausibly give rise to heightened vulnerability and symptoms. Once a candidate endophenotype has been selected, it must be scrutinised in particular circumstances to determine whether it fulfils the minimum criteria of (1) association with relative risk for illness and (2) heritability. Association with illness-risk can be determined by correlating the endophenotype with genetic proximity to affected individuals, and also by identifying

the endophenotype in individuals with schizophrenia-spectrum personality features (see Chapter 3 for further discussion of the spectrum concept). Heritability of the endophenotype in the general population and in families affected by psychiatric illness can be assessed in twin and sibling pairs using classic behavioural genetic techniques. If an endophenotype has been found to be reliably associated with illness-risk, it can be used to elucidate causal factors that influence individual differences in the developmental process, as indexed by variation in endophenotype.

1.4.2 The endophenotypes approach and gene-hunting in schizophrenia

Many potential traits that are consistently found to be abnormal in patients have been investigated in family members to assess the degree to which they are associated with schizophrenia risk rather than with the expression of the illness. However, only a small number of studies have extended descriptive results to capitalise upon neurophysiological or cognitive traits as tools for genetic analysis. A good example of this approach is the detection of co-segregation of P300 (an auditory event-related potential component) with a translocation at 1q42 disrupting two large genes of unknown function (DISC-1 and DISC-2) within a family multiply affected by both schizophrenia and other psychiatric illnesses (Blackwood et al., 2001). The neural marker co-occurs with the translocation regardless of the presence or absence of psychiatric illness i.e. all symptomatically affected and some unaffected family members show both the translocation and a P300 abnormality. Unfortunately, screening for predisposition alleles within these genes in unrelated schizophrenia samples has so far indicated that this gene is unlikely to contribute to risk in the general population (Devon et al., 2001). Nevertheless further investigation of this family may identify molecular, neurobiological and developmental pathways contributing to P300 abnormality and schizophrenia risk that are potentially informative for the general population.

A second approach informed by the endophenotype concept is to conduct linkage studies within a larger sample of families multiply-affected by schizophrenia, using an endophenotype rather than categorical diagnosis to assign affected / unaffected status. A powerful study, conducted in Finland, has linked quantitative abnormalities in cognitive function in discordant twin pairs to a locus at 1q (Gasperoni et al., 2003). No

candidate gene has yet been identified from this study. Using a neurophysiological measure (abnormalities in gating the P50, a short-latency auditory ERP), Freedman and colleagues (1997) identified a risk-associated dinucleotide polymorphism in the nicotinic acetylcholine receptor at 15q13-14 by linkage and association methods. A subsequent study of the combined effect of several polymorphisms in the promotor region of this gene, found a strong association between the presence of polymorphisms and the P50 abnormality in a control population with no family history of schizophrenia. However both the risk-related genetic and endophenotypic factors were prevalent (~30%) in this general population sample, and the contribution of either the genetic variant or the endophenotype to diagnosis was small (Leonard et al., 2002). Either this gene and its associated physiological deficit contribute to risk for schizophrenia to a small degree, acting alongside many other factors, or these findings are epiphenomena unrelated to schizophrenia, most likely reflecting susceptibility to nicotine addiction which is prevalent both in the patient and control populations. Use of other potential markers of inhibitory processing such as pre-pulse inhibition of the auditory startle reflex and smooth pursuit eye movements have proved less fruitful in genetic analysis to date, although these psychophysiological parameters are found to be abnormal in patients and their healthy relatives and in the case of PPI have been associated with genetic variation and specific neurotransmitter systems in mice (Cadenhead, Swerdlow, Shafer, Diaz, & Braff, 2000).

Another manifestation of the endophenotypes approach, pioneered by Weinberger and colleagues, it to begin with a candidate gene already associated with risk for schizophrenia, and to examine an allelic variant in this candidate as a potential determinant of schizophrenia-related cognitive and neural abnormalities in patients, family members and unrelated individuals. The most notable example of this type of study to date has been the search for cognitive correlates of a functional variant in the *catechol-o-methyl transferase (COMT)* gene, which lies within the 22q11DS region of chromosome 22 (Weinberger et al., 2001). These findings and their implications for 22q11DS will be discussed in Chapter 8.

Several studies are now aiming both to replicate findings and extend the application of these cognitive and neurophysiological methods in larger populations, and to increase the number of potential endophenotypes under examination at any one time. Conducting these investigations alongside large-scale genome analysis may yield more sophisticated

data on the co-ordinated architecture of genes contributing to neurodevelopment and to risk for illness, and may be able to address the issue of genetic and neurobiological heterogeneity within the schizophrenia diagnosis (Holden, 2003). Nonetheless smaller scale studies of high-risk populations (including 22q11DS), continue to be necessary, to define new neurocognitive abnormalities for investigation and identify potential genetic influences.

1.4.3 Applying the endophenotypes approach to 22q11DS

As discussed above, schizophrenia risk-related disruption is present prior to the onset of severe psychotic symptoms, and manifests itself throughout the lifespan as a spectrum of atypical characteristics, reflected in endophenotypes as well as overt features. These general principles may also apply in 22q11DS, but this has not yet been empirically established. There are likely to be many different neurodevelopmental routes towards schizophrenia, and multiple components within the individual which interact at different points in time and at different levels (molecular, neurochemical, neurocognitive and psychological) to increase or reduce the likelihood of symptom expression. The particular mechanism of genetic and molecular disruption in 22q11DS and its neurodevelopmental and psychiatric manifestations may be shared with at least a subset of individuals with idiopathic schizophrenia, or the pathway may be unique to the syndrome. Obtaining evidence for overlap in specific neurocognitive risk factors for psychotic illness in 22q11DS and in idiopathic schizophrenia, as indexed by endophenotypes, was the primary aim of this study.

By studying developmental and functional processes in 22q11DS for which there is existing evidence of disruption in association with schizophrenia in the general population, our understanding of these processes, and their status as potential endophenotypes for other high-risk and genetic studies, may be enhanced. In the current study, potential endophenotypes were selected for evaluation in 22q11DS on the basis of (1) existing evidence of genetically-mediated developmental dysfunction in schizophrenia, (2) reasons to predict abnormality in the syndromal group independent of schizophrenia-risk, and (3) a priori interest in phenomena that promote the establishment of integrated models of psychosis that can accommodate information obtained from multiple levels of analysis.

The study design rests on the principle that variation within the index group (22q11DS) is as important as defining differences between groups (22q11DS and control populations), and that by investigating within-group variation at different levels of analysis, mechanisms and mediating factors can be identified. It is not possible to directly determine which gene within a relatively large deleted region of the genome is influencing development, unless a point mutation or chromosomal disruption (for example a translocation breakpoint within a coding region) is detected within a candidate gene, in association with expression of a phenotype or endophenotype. In the absence of such informative cases, it is only possible to infer which gene within the deleted region might be influencing neurodevelopment and relevant aspects of brain function by investigating the impact of polymorphisms in potential genes of interest on within-sample variation in expression of associated features.

Several candidate risk genes for neuropsychiatric disruption exist within the 22q11 typically-deleted region, identified either on the basis of linkage or association findings in psychiatric populations, or functional candidacy. Inferential evidence of a role for one candidate gene, *catechol-o-methyl transferase* (*COMT*), selected for all of the above reasons, will be sought, by sequencing a known functional polymorphism in this candidate gene, on the lone non-deleted chromosome 22 in 22q11DS cases. The allelic variant in this candidate gene ($COMT^{val}158^{met}$), which has been shown to influence schizophrenia-relevant cognitive function in the general population, will be tested as a potential factor impacting upon the expression of endophenotypic features and / or overt psychiatric symptoms within the 22q11DS population (Chapter 8).

1.4.3.1 Selection of candidate endophenotypes

Description and justification of each measure chosen for evaluation as a potential endophenotype for psychotic illness in 22q11DS is presented in the relevant chapters (Chapter 4 for cognitive function, Chapter 5 for basic auditory processing, Chapter 6 for speech processing). The process by which other potential endophenotypes were excluded from this investigation is briefly reported below.

Neuropsychological tests were excluded if performance was likely to be influenced by non-specific ability factors such as comprehension and attention, since most study participants in this investigation have mild or moderate learning disability. Tests known

to stress multiple cognitive skills were also avoided. For both these reasons, the Wisconsin Card Sorting Test, a test of executive function widely used in schizophrenia research, was not employed. The Continuous Performance Test, a simple test of sustained and selective attention with good credentials as a potential endophenotype for schizophrenia (Cornblatt & Malhotra, 2001) was piloted in several control participants but excluded from investigation because the patience-trying nature and length of the task led to participants discontinuing the test prior to its conclusion.

Several neurophysiological measures that have previously been utilised as endophenotypes in schizophrenia research were considered and then ruled out. Pre-pulse inhibition (PPI) is a commonly-used measure which is conceptually and practically simple, indexes function of a well-characterised neural circuit and has the additional benefit of being easily investigable in mice (Braff, Geyer, & Swerdlow, 2001). Although the baseline magnitude of the startle reflex varies enormously between individuals (Abel, Waikar, Pedro, Hemsley, & Geyer, 1998), reasonable test-retest reliability has been demonstrated for PPI in both normal adults (Cadenhead, Carasso, Swerdlow, Geyer, & Braff, 1999) and schizophrenia patients (Ludewig, Geyer, Etzensberger, & Vollenweider, 2002).

The evidence that deficient PPI is a direct reflection of a vulnerable process in schizophrenia is ambiguous. Using a passive paradigm, Braff and colleagues detected deficits in acoustic and tactile startle eyeblink suppression, at short and long pre-pulse to pulse intervals, interpreted as a pan-modality impairment in automatic sensorimotor gating (Braff, Grillon, & Geyer, 1992). However in a series of studies Dawson and colleagues failed to replicate this finding, with schizophrenia patients showing impaired suppression of the eyeblink only at long (120ms) inter-pulse intervals and only when required to actively attend to the pre-pulse (Dawson, Schell, Hazlett, Nuechterlein, & Filion, 2000). This study showed that, in control populations, attending to the pre-pulse causes additional suppression of the startle eye blink whilst this attentional modulation effect is not seen in schizophrenia patients. Dawson et al therefore conclude that controlled cognitive processes, rather than sub-cortical automatic gating deficits, underlie PPI deficits in schizophrenia. The startle reflex can be modulated by other non-constant factors including emotional state, suggesting that it is a motor outcome influenced by many different cognitive processes (Filion, Dawson, & Schell, 1998).

These modulatory cognitive processes may be more directly relevant to psychosis than the basic startle response and its automatic gating.

An additional factor that may explain contradictions between studies is that Braff et al studied relatively old patients with long histories of medication use and hospitalisation whilst Dawson et al studied relatively asymptomatic outpatients. This suggests that intact PPI could predict better outcomes within schizophrenia populations rather than trait vulnerability, or that deficits may emerge as a consequence of chronicity. Family studies have indicated that PPI deficits are found in association with susceptibility to psychosis (Cadenhead et al., 2000) and therefore may be useful in future genetic studies. However startle modification deficits have also been observed in other clinical populations, most notably in anxiety disorders (Filion et al., 1998). It is plausible that so-called genetic effects on PPI could reflect different affective state during experimental sessions, since the emotional context of taking part in schizophrenia research will not be equivalent for relatives of patients and control individuals. An additional consideration with regard to the use of PPI is that it requires the presentation of extremely loud auditory stimuli (loud enough to illicit a startle, akin to unexpectedly hearing a balloon bursting). It is thus an unpleasant procedure, and its use is ethically dubious, especially in children.

Another technique for eliciting information about sub-cortical motor control processes is assessment of eye movements as measured by infrared occulography (e.g. smooth-pursuit tracking and inhibition of anticipatory saccades). There is some evidence that these phenomena may index genetic liability to psychosis in both adults (Ross et al., 2002) and children (Ross, 2003) but as yet they have not been directly employed as markers for genetic analysis. Although eye-movement measurement has been performed in paediatric populations, the tasks are effortful to perform and therefore may be unreliable in young and learning disabled populations. This method requires highly specialist equipment and skills that were not locally available for the current study. In a similar fashion to PPI, eye-tracking abnormalities are indirect observations of atypical cognitive processes and higher-level cortical operations of relevance to psychiatric well-being. If these processes can be more directly investigated then results may be more reliable and informative of subtle neurobiological abnormalities.

It is perfectly plausible that the techniques described above would reveal interesting information about schizophrenia-like differences in 22q11DS, possibly even being informative of mechanisms of neurobiological disruption. For the current study, however, neurophysiological measures were selected for which reliability could be demonstrated in preliminary test-retest experiments, which did not require specialist equipment and skills, and which did not rely upon accurate performance of a task. These criteria should ensure that results are robust, even in a young population with learning disability, thus facilitating valid genetic analysis of small subgroups of the total study population. The final consideration with regard to selection of methods was that experiments should be developed and carried out within the framework of contemporary cognitive neuroscience, using concepts and techniques that are applied by many neuroscientists in investigations of normal neurocognitive function and development, and that are not predominantly utilised in psychiatric research as markers of pathophysiology. This rich experimental and theoretical context should enable findings to be more easily integrated into models of typical and atypical neurocognitive development and psychological experience.

1.5 Aims of this study

- to improve the characterisation of developmental psychopathology in 22q11DS.
- to identify neurocognitive processes that are vulnerable to disruption in 22q11DS and that may explain the high rates of psychotic illness displayed by this population.
- to explore the relationship between neurocognitive disruption and the emergence of psychiatric symptomatology in adolescents and young adults with the syndrome.
- to define markers of neuropsychiatric vulnerability (endophenotypes) as tools to identify the gene(s) within the deleted region of chromosome 22 responsible for mediating elevated risk of psychiatric illness in the 22q11DS population.
- to apply these tools to the identification of a critical gene mediating neuropsychiatric vulnerability within the 22q11DS population.

1.6 Summary

Evidence from diverse disciplines has contributed to the establishment of a neurodevelopmental hypothesis for schizophrenia. Investigation of high-risk groups confirms the existence, and assists in the delineation, of specific disruptions prior to the onset of illness, which can be observed either as overt behavioural phenomena or more subtle latent traits. However high-risk studies are marred by population heterogeneity and have focused on dichotomisation of subjects into "affected" and "unaffected" groups on the basis of diagnostic outcome. The endophenotypes approach - investigating continuous liability indicators that directly reflect the action of risk genes as regulators of normal developmental and functional processes – is promising as a strategy for identifying potential genes of interest, for understanding their mechanism of action, and for approaching gene-environment interactions throughout development.

It is reasonable to posit that schizophrenia (or some similar psychiatric disturbance) in 22q11DS comes about because one or more genes in the typically deleted region are involved in regulating aspects of brain development, such that haploinsufficiency renders these developmental processes vulnerable, giving rise to very much increased risk relative to the general population. Given the lack of direct strategies to determine the causative gene(s) for cognitive and psychiatric impairment in this syndrome, identifying endophenotypes that can be used as markers of relative vulnerability may be worthwhile. In common with other developmental disorders, 22q11DS offers an opportunity to move beyond "genes for behaviours" to "genes for the regulation of developmental and functional neurocognitive processes that are relevant to specific aspects of cognition and mental health". Narrowing the arena of investigation, from an association between the microdeletion and illness outcome, to associations between specific genes and neurocognitive processes, could assist in future investigations of pathophysiological mechanisms, in animal models, and in this and other human populations.

2 Study populations

2.1 Introduction

Adolescents and young adults were selected for study, specifically in order to assess subjects prior to the time of peak likelihood of onset of psychosis. All assessments were carried out in comparison with age- and IQ-matched subjects, without a history of psychosis, or any known genetic anomaly. Comparison to an IQ-matched control group is necessary to account for the non-specific effect of neurodevelopmental abnormalities, associated with learning disability, on neurocognitive and psychiatric variables. Exclusion of individuals from the control group who have a history of psychosis removes the potential confound of overlap in endophenotype status that may exist between 22q11DS and individuals with a psychotic illness.

To determine the specificity of atypical developmental features for 22q11DS and for psychiatric rather than cognitive risk, additional comparisons should be conducted with other developmental disorders, either defined by genetic anomaly or by behavioural characteristics. In groups not known to share psychiatric risk with 22q11DS but with some degree of similarity in cognitive or behavioural profile (e.g. Fragile X syndrome, Attention Deficit Disorder) overlap in endophenotypes would not be expected. A comparison between 22q11DS subjects and children with Specific Language Impairment (SLI) was conducted as part of the current study, both to extend the neurophysiological characterisation of SLI, and in order to begin to address the issue of endophenotype specificity in 22q11DS. 22q11DS individuals commonly display speech, language and communication impairments, the hallmarks of SLI. The routes towards language impairment in 22q11DS and SLI (which are also highly likely to be heterogeneous) may or may not be distinct, and thus the two groups may show similar or different endophenotypic features.

2.2 *Sample recruitment*

2.2.1.1 Ethical approval

This study received ethical approval from the Great Ormond Street Hospital NHS Trust research ethics committee (project reference 97BS12). All participants and parents / carers were provided with information sheets explaining each part of the study. Written informed consent was obtained from parents / carers, and from participants aged over 16.

2.2.1.2 22q11DS group

22q11DS subjects were recruited with the assistance of the UK 22q11 Group (a nationwide family support and information group) over a four-year period, initially to take part in a questionnaire-based study of childhood behavioural function in 22q11DS. Information about this study was provided to parents of individuals diagnosed with the 22q11 deletion at a multi-disciplinary clinic held at the Chelsea and Westminster Hospital, and via the newsletter and website of the UK 22q11 Group. A total of 150 young people were recruited to the questionnaire study between 1997 and 2002.

Parents who had taken part in the questionnaire study, or who had expressed interest in taking part in research, and whose son or daughter was aged 13 and over at the start of 2002, were contacted by means of letter with information about the current study. This was a total of 48 individuals of whom 32 expressed interest in taking part by returning a reply slip or telephoning the department. Of those who expressed interest, 5 chose not to take part in the study once further details were supplied over the telephone. Two individuals took part in psychiatric assessments only. One was excluded from cognitive testing because of severe learning disability secondary to post-operative brain damage, the other because of absent expressive language.

2.2.1.3 Comparison subjects for 22q11DS group

Controls were recruited from schools, colleges of further education, and leisure organisations providing services for young people with learning disabilities. Special needs co-ordinators, course directors and youth workers were asked to select young people with low average general ability of mild / moderate learning disability but no known neurological or genetic condition or major sensory impairment. Psychiatric disorder or behavioural problems were not exclusion criteria since this would have

biased the psychopathological results towards an unrepresentative low rate of disruption in controls. The parents / carers of selected individuals were contacted by letter, and expressed interest in the project by returning a reply slip or telephoning the research department. Sources of referral were mainstream schools (64%), FE colleges (12%) and a special needs sports club (24%).

2.2.1.4 Specific Language Impairment group

SLI children were recruited via specialist language centres and resource bases across the Midlands and South West of the U.K., with a clinical diagnosis of primary language impairment. Stringent screening was carried out to select a group with impairments in receptive and expressive language but not articulation or pragmatics. For inclusion in this study, children were required to meet three of the following criteria: Verbal Comprehension index score of 80 or less from the Wechsler Intelligence Scale for Children-III (Wechsler, 1992); Repeating Sentences scaled score of 6 or less from Clinical Evaluation of Language Fundamentals (Semel, Wiig, & Secord, 1987); Test for Reception of Grammar scaled score of 85 or less (Bishop D.V.M., 1989); Children's Test of Nonword Repetition scaled score of 80 or less (Gathercole, Willis, Baddeley, & Emslie, 1994). Additional inclusion criteria were WISC-III Performance IQ of above 80, or a statistically significant (>15 point) discrepancy between WISC-III Performance and Verbal IQ scores. Children with hearing impairment, neurological or genetic syndromes were excluded, as were children with pure articulation deficits. Children with pragmatic language impairment, as defined by scores less than 132 on the Children's Communication Checklist (Bishop, 1998), were also excluded, since these impairments may be indicative of an autistic spectrum disorder.

2.2.1.5 Comparison subjects for SLI group

Children were recruited to the SLI comparison sample from a variety of sources including local schools, academic colleagues and hospital staff. This group was only selected to match the SLI group on age. Assessments of nonverbal intelligence were carried out to ensure there was no difference in general cognitive ability between groups. Standard exclusion criteria of sensory, neurological or specific learning impairment also applied.

2.3 *Measures of general ability*

2.3.1.1 22q11DS study

Abbreviated IQ tests were administered in order to obtain estimates of general ability in minimal time. For participants aged 16 years and over (n=18), a sub-test quartet from the Wechsler Adult Intelligence Scale – Revised (Wechsler, 1981) consisting of Comprehension, Similarities, Block Design and Object Assembly was administered. Scaled scores were summed and an estimate of full scale IQ calculated according to British adult normalised data (Crawford, Allen, & Jack, 1992). For participants under the age of 16 (n=32), a sub-test quartet from the WISC–III^{UK} (Wechsler, 1992) consisting of Vocabulary, Similarities, Block Design and Picture Arrangement was administered, and converted to an IQ estimate according to published equations (Kaufman A.S., Kaufman, Balgopal, & McLean J.E., 1996). There was no significant difference between mean IQ estimates obtained using the WAIS-R (mean 73, s.d. 8.7) and WISC-III tetrads (mean 68.3, s.d. 18.5) ($t = -1.2$, $p=0.3$).

2.3.1.2 SLI study

The SLI group was compared to a typically-developing age-matched sample on estimates of nonverbal intelligence. These were assessed either by administering WISC-III subtests to obtain a performance IQ score, or by administering Raven's Standard Progressive Matrices (Raven, Raven, & Court, 1998).

2.4 Group characteristics

2.4.1.1 22q11DS study

22q11DS and control subjects were well matched for gender. The minimum age of study participants was 13, and the maximum 23, in both groups. There was a slight trend towards the lower end of the age-range in control subjects; it was more difficult to recruit young adults with mild learning disability from the community, than to identify 22q11DS individuals within this age range. There was also a trend towards higher estimated full-scale IQ in the control group, although this did not reach statistical significance. Identifying individuals with IQ in the 50-70 (moderate learning disability) range who do not have a genetic diagnosis or evidence of congenital syndrome, no neurological impairment and no specific learning disability (e.g. autism) is very difficult. It is arguable whether such a group really exists, since IQ in this range signifies some degree of neurodevelopmental disruption, which must have an aetiological origin of some type or other. It is likely that the individuals within the control group of lowest IQ do in fact have some unidentified cause for their poor general cognitive ability, be this genetic or developmental. Nevertheless, the groups can be considered to be matched on estimated cognitive ability, such that any group differences can be ascribed to neurodevelopmental disruptions that are independent of learning disability in 22q11DS.

Table 2.1 22q11DS and control groups – gender, age and IQ

	Group		t-value / chi-square
	22q11DS (n=25)	**Controls (n=25)**	
Gender (male : female)	15:10	17 : 8	0.35
Age (years)	16.4 (2.0)	16.0 (2.9)	1.2
Estimated IQ	66 (15)	74 (16)	1.9

2.4.1.2 22q11DS event-related potential study

A subset of the case-control sample described above took part in an EEG session at Great Ormond Street Hospital. This subset was representative of the entire sample, and well matched, in terms of age, estimated full-scale IQ and gender. Non-participants were excluded because of the presence of hearing impairment, or declined to take part because of the need to travel to London for the testing session.

Table 2.2 22q11DS and control groups (ERP study) – gender, age and IQ

	Group		t-value / chi-square
	22q11DS (n=16)	**Controls (n=16)**	
Gender (male : female)	9 : 7	12 : 4	1.2
Age (years)	16.5 (2.7)	15.6 (2.4)	1.0
Estimated IQ	67 (15)	76 (17)	1.5

2.4.1.3 SLI study

Groups were well matched for age and gender. There was a trend towards lower nonverbal IQ in the SLI group relative to controls. This reflects the possibility that SLI is not absolutely specific to language in all cases, and that deficits in some performance IQ tasks are also commonly found. This may in part be a confound due to the linguistic content of some Wechler performance IQ sub-tests, for example picture arrangement which requires sequencing picture cards into a story. Another explanation for low performance in the SLI group on nonverbal tasks is the need for verbal comprehension of task instructions.

Table 2.3 SLI and control groups – gender, age and IQ

	Group		t-value / chi-square
	SLI (n=12)	**Controls (n=12)**	
Gender (male : :female)	7:5	6:6	0.17
Age (years)	10.5 (1.2)	11.0 (1.9)	0.7
Estimated non-verbal IQ	91 (14)	100 (21)	1.2

3 Psychopathology

3.1 Introduction

3.1.1 Aims of psychopathological investigation

A case-control comparison of psychiatric status in adolescents with 22q11DS was conducted as part of this project for several reasons. Firstly, there has to date been no systematic study of the psychological features of 22q11DS during adolescence and young adulthood. Such a study would clarify the spectrum of types and severity of atypical psychological function in this group, providing information for parents and professionals regarding syndrome-associated features. Inclusion of an age and IQ-matched comparison group may point towards similarities and differences between the behavioural phenotypes found in association with 22q11DS and other developmental disorders, and define a spectrum of psychopathological disruption that is specific to this syndrome.

Secondly, identification of syndrome-specific atypicalities in psychological function in the form of specific symptoms and symptom-profiles might suggest mechanisms underlying neuropsychiatric risk and generate hypotheses for future neurocognitive research. As previously discussed, schizophrenia is an extremely heterogeneous illness in terms of the number and type of symptoms that can lead to a diagnosis and the developmental progression which might lead towards its emergence in any individual. Some authors have argued that emotional disruption lies at the heart of the emergence of the illness in early adulthood, and that this emotional component is later overshadowed by psychosis and cognitive impairment secondary to illness-related deterioration (Birchwood, 2003). Other contemporary theorists suggest that psychosis itself is the core of the disorder, arising from a pre-existing cognitive bias towards atypical perceptual experiences (Kapur, 2003) or abnormal metacognitive attributions for example aberrant self-monitoring and awareness of agency (Frith, Blakemore, & Wolpert, 2000). The symptom profile in 22q11DS adolescents may point to one or other core neuropsychiatric mechanism, or a novel pathway, which could then be investigated at a neurocognitive level.

The relationship between schizophrenia-spectrum disruption / premorbid features and neurocognitive development is an area of active investigation at present. Combinations of different types of vulnerability markers (subjective and objective) may increase the specificity with which predictions can be made within high-risk and ultra-high risk populations (Cadenhead, 2002). Similarly, sub-typing schizophrenia by neurobiological traits rather than by behavioural syndromes has often been proposed as the way forward in effective treatment of the illness, although obtaining data to this end is difficult. Many studies have attempted to find cognitive or neural correlates of specific dimensions of schizotypy in both patients with schizotypal personality disorder and in psychometrically-defined populations but results have been very inconsistent, as reviewed by Suhr & Spitznagel (2001). Study of spectrum-disordered individuals or high-risk individuals from this perspective is important, because it removes the confounding factors of medication status and chronicity on psychiatric and functional deterioration, which are likely to both increase symptom scores and reduce performance on neurocognitive tests in an associated but non-specific manner.

A third reason for conducting this descriptive exercise is that within-sample variation in psychiatric function during adolescence may be a marker of relative neuropsychiatric risk in the 22q11DS group. The clinical concepts of premorbid adjustment, schizotaxia, schizotypy and the prodrome to schizophrenia (defined below), alongside the empirical determination of their properties within patient samples, their relatives and the general population, suggest that schizophrenia refers to a continuum of illness rather than a categorical, qualitative distinction in two respects. Firstly, the progression from "premorbid" state to onset of illness may be continuous (in the form of long-term deterioration) and also ongoing (in the form of repeated relapses and remissions). Alternatively, psychosis-related phenomena may emerge without psychological and behavioural forewarning. Secondly, the distinction between schizophrenia and less severe but qualitatively similar conditions, now termed schizophrenia-spectrum disorders, may be continuous within the general and high-risk populations. On the other hand, a categorical subgroup of individuals with 22q11DS may be vulnerable to psychosis and display marked premorbid features whilst the remainder display no such phenomena. Assessments during adolescence will partially address these two issues, and provide a psychiatric "baseline" for future longitudinal studies of the progression of psychological dysfunction towards psychosis in 22q11DS which will provide more definitive conclusions.

3.1.2 Psychopathology in the pre-schizophrenia phase

High-risk studies have indicated that abnormal behavioural and social adjustment through childhood and adolescence are associated with increased likelihood of illness in adulthood (Amminger et al., 1999). Similarly, follow-back studies of adult patients have shown consistent results based either on population-based cohort information (Done, Crow, Johnstone, & Sacker, 1994), child psychiatric case notes (Hollis, 2003) and retrospective interview of relatives (Cannon et al., 1997). The general finding is that adults with schizophrenia deviated from their peers in psychosocial functioning at a very early age, the most commonly reported abnormalities being social withdrawal, anxieties and attention or learning problems at school. The same abnormalities were not present to the same degree in adults with bipolar disorder or non-psychotic disorders. Debate continues as to whether this represents quantitative variation in degree of premorbid adjustment difficulties (Cannon et al 1997), or a qualitative distinction between the types of early impairment predictive of outcome. There is some evidence to suggest that, unlike cognitive features, psychological traits during childhood differentiate between siblings discordant for adult outcome, since observers blind to adult diagnosis can reliably predict which sibling will develop schizophrenia from home movies (Walker & Lewine, 1990). This suggests that these traits represent environmentally mediated expression of underlying vulnerability which may then itself act as a developmental risk factor to increase the likelihood of manifesting the illness. In 22q11DS, stable or progressively abnormal psychological function during childhood and adolescence may predict poor psychiatric outcome, but this can only be assessed longitudinally.

Three different, to some degree overlapping, concepts that have been defined in relation to psychological indication of psychosis-risk are described below. These are premorbid adjustment, schizotypal personality and the schizophrenia prodrome.

Premorbid adjustment (PMA) is a very broad term referring to poor or deteriorating social and adaptive functioning prior to the onset of psychosis. Some authors have argued that this pre-psychotic state in fact resembles a developmental "negative" syndrome continuous with that experienced following onset of psychosis and there is some evidence for this from longitudinal analysis of psychiatric case notes from pre- and post-psychotic phases (Hollis 2003). Poorer PMA and more severe negative

symptoms during the early years of a schizophreniform illness are associated with a more chronic and disabling illness, suggesting that they reflect either a different or more severe neurodevelopmental course (Fenton & McGlashan, 1991). A similar suggestion is that these early and continuous symptoms represent the behavioural component of schizotaxia, the familial neuropsychological and clinical state that may progress in some individuals to either schizotypal personality disorder or schizophrenia (Stone et al., 2001). Within an at-risk group (including 22q11DS), individuals at highest risk for schizophrenia due to more severe neurodevelopmental disruption would be predicted to display poorer premorbid adjustment.

The component features of the psychopathological spectrum (or spectra, since there may be multiple distinct dimensions that are present in some but not all individuals displaying features) have been described within the framework of a set of personality traits termed schizotypy. Several questionnaire and interview-based measures of schizotypy have been developed in order to quantify an individual's personality on dimensions such as magical and paranoid thinking, abnormal perceptions, and cognitive, sensory and social phenomena (Diduca D & Jospeh S, 1999). Some but not all factor analyses have suggested continuity between schizotypal dimensions and symptom clusters (positive, negative and disorganised / social-affective) in schizophrenia. Population based studies have found a broad distribution of schizotypal scores indicating a continuous distribution of psychological risk / liability to schizophrenia. A similar concept has been proposed by Van Os and colleagues with regard to subclinical psychosis-like phenomena. They have found in several large population-based studies that the experiences of mild hallucination and delusional thinking are distributed fairly widely in the general population, again challenging the absolute distinction between the mental experiences of the psychotic and non-psychotic individual (Verdoux & van Os, 2002). These results remain controversial within the psychiatric community since they are perceived by some as challenging the rational basis for diagnosis. However with regard to both the genetic and neurobiological underpinning of psychotic illness these studies are important in indicating the existence of continuous liabilities of risk.

The prodromal syndrome is a collection of observable changes in psychological function and behaviour in the months or years leading up to a psychotic episode. It comprises features that overlap with both PMA and schizotypy - attenuated positive

symptoms, affective instability and deterioration in social and behavioural function. There has been much interest in recent years in defining criteria for the prodromal syndrome in order to reliably identify schizophrenia "cases" prior to the onset of psychosis and to institute preventative interventions. Two instruments for determining the presence of the syndrome have recently been published, the SIPS (Structured Interview for Prodromal Syndromes, Miller et al (1999)) and the CAARMS (Comprehensive Assessment of the At-Risk Mental State, Phillips et al (2002)) alongside criteria for diagnosis. The predictive power of these instruments clearly varies depending on the population included for investigation. At the population level, the ability of the prodromal syndrome to predict cases of schizophrenia would be extremely low – McGorry et al (1995) found that one or more DSM-III-R prodromal features were present in 50% of high school adolescents. Within a referred population of young people suspected to be at high-risk for psychosis either on the basis of family history or symptoms, predictive power for transition to diagnosis is only moderate: 54% at 12 months for the SIPS (Miller et al., 2002) and 41% for the CAARMS (Yung et al., 2003). Given the evidence for a continuum of disruption from schizotypal liability to disabling schizophrenia this low rate of "conversion" is not surprising. An ethical question taxing many researchers and clinicians is the benefits and risks of offering therapeutic interventions to young people showing prodromal signs. In addition to PMA characteristics and schizotypal personality features, it was predicted that some adolescents with 22q11DS may display psychiatric symptoms similar to the prodromal syndrome –.

3.1.3 Previous studies of psychopathology in children and adolescents with 22q11DS

The very first description of psychological function in a group of children with clinically-defined VCFS, stated that "the impression was notably different from that of the personalities of other syndromes associated with intellectual impairment" (Golding-Kushner, Weller, & Shprintzen, 1985). The features emphasised were bland affect, minimal spontaneous facial and vocal expression, poor social interaction and a tendency towards extremes of behaviour. These features were felt to be "strikingly characteristic". Whilst this early study used neither standardised assessments nor a control population, it set the stage for future investigations as it posited the existence of a unique and characteristic pattern of difficulties (by definition, therefore, a behavioural

phenotype). Swillen and colleagues claimed to confirm the initial sketch formulated by Golding-Kushner (Swillen et al., 1997) by assessing a relatively small group of children with the Child Behavior Checklist (Achenbach, 1991). However, when comparison-data was obtained for children with idiopathic developmental delay, no group differences on either total or subscale (for example anxious / depressed, aggressive) scores were detected (other than some very marginal increases in aggressive behaviours amongst controls) (Swillen, Devriendt, Ghesquiere, & Fryns, 2001). This negative finding was replicated by another group using the same instrument and similar populations (Feinstein, Eliez, Blasey, & Reiss, 2002).

The first study to conduct standardised (parental) psychiatric interviews in a paediatric 22q11DS population (Papolos et al., 1996) assessed individuals within a relatively wide age-range, with no control population. They detected a remarkably high rate of bipolar disorder (16 of 25 participants), with psychosis and other schizotypal features evident in the oldest 4 subjects. This led to the suggestion that the 22q11 locus harboured a high-risk gene for psychotic disorders, rather than for schizophrenia specifically, with evolving behavioural expression across the life-span. Later studies (Arnold, Siegel-Bartelt, Cytrynbaum, Teshima, & Schachar, 2001; Feinstein et al., 2002) have reported high rates of a very wide variety of disorders, in particular ADHD and oppositional behaviours, phobias, anxieties and mood disorders. Arnold et al reported significantly more mood disorders in comparison with sibling controls, but this was not replicated by Feinstein et al in comparison with a more appropriate IQ-matched group. Lastly, Niklasson and colleagues (2001) detected an autism-spectrum disorder in almost one-third of children and young adults with the microdeletion, although this may not be an elevated rate for children with moderate learning disability depending on the specific criteria used for diagnosing social communication disorders.

There are several limitations to existing studies of psychopathology in children and adults with 22q11DS. Firstly, subjects from wide age-ranges have typically been combined into a single group, unsurprising given the small number of available subjects. This may mask age-dependent, syndrome-specific characteristics. Secondly, only two studies have compared psychological features in 22q11DS with developmentally appropriate control groups. This is necessary to determine whether any indicators within the group that may predict adult psychiatric outcome are specific to the syndrome and are not general indications of learning disability in conjunction

with poor psychosocial adjustment. Thirdly, no published study has yet associated atypical psychological features with specific neurocognitive abnormalities. Fourthly, interview-based assessments have been carried out with parents only, without the supply of additional information from the young person themselves or from additional sources such as teachers. Single-informant assessments are notoriously unreliable (Jensen et al., 1999) and may be particularly so during adolescence and in relation to subjective experiences such as psychosis.

These concerns will be partly overcome in the current study, although the limitations of small study size and of fairly wide age-band remain. An idiopathic learning disability group was investigated alongside 22q11DS subjects. Information was sought from both parents / carers and index young person in the majority of both case and control groups. The use of multiple informants increases the likelihood that symptoms will be reported, but introduces the problem of inter-informant disagreement. This is a particular problem during adolescence, when agreement between parents and teenagers tends to be very low. In addition, subjects with learning disability may have difficulty in understanding and responding to complex questions relating to emotional and mental states and requiring detailed information about timing and frequency of events. Thus an inclusive approach was taken to the coding of symptoms - the young person more often provided information pertaining to the nature and content of symptoms, but detailed information about frequency, duration and severity of disruption was more often obtained from the parent. This approach is in line with method found to be acceptable for combining information from multiple informants for childhood psychiatric diagnoses, in comparison with other, more complex statistical techniques (Bird, Gould, & Staghezza, 1992).

3.1.4 Hypotheses

On the basis of the concepts and evidence presented above, four hypotheses were proposed with regard to schizophrenia-related psychopathology in adolescents with 22q11DS:

1. 22q11DS subjects display more psychiatric symptomatology than IQ- and age-matched controls. Symptoms may include non-specific psychological, social and functional impairment (reflected in multiple DSM-IV diagnoses and poor premorbid adjustment) and / or schizotypal features (specifically reflecting psychosis-proneness).

2. Psychiatric symptoms and rates of diagnoses are independent of IQ in 22q11DS, since previous studies have not found any relationship between learning disability and mental illness in this group.
3. Psychiatric symptomatology increases with age in 22q11DS, reflecting deterioration in function in early adulthood similar to the prodromal syndrome in idiopathic schizophrenia.
4. A relationship should exist between psychiatric symptoms and behavioural characteristics such that individuals with high levels of schizotypal personality disorder and prodromal symptomatology will display poorer social function and occupational performance (as assessed by PMA scales).

3.2 Method

3.2.1 Psychiatric Interview

A semi-structured interview of general psychopathology, the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 1995) was selected as the primary instrument for obtaining a descriptive record of each subject's current (previous 3 months) mental state. The CAPA has been found to have reasonable test-retest reliability and to correspond well with other measures of childhood psychiatric disorders. This instrument was selected because of its dimensional structure, its dependence on behavioural examples for the coding of symptoms which assists clinically inexperienced interviewers in correctly assigning scores, and because of the local availability of a training course and regular coding meetings with one of the authors of the instrument. Prior to the start of the investigation, practise interviews were conducted with 3 community-recruited subjects with and without learning disability, and 3 inpatients in an adolescent psychiatric unit. Practise interviews were discussed with two highly experienced child psychiatrists and consensus symptom coding and diagnoses reached.

For all subjects in the case-control study both the young person and one or two parents / carers were interviewed using the appropriate CAPA schedule. Interviews took place at the participants' home, except for four 22q11DS subjects and one control who were assessed at Great Ormond Street Hospital. Whenever possible, parents and children were interviewed separately. However in several cases this was not possible, because either the parent or the young person preferred to remain together. In these

circumstances, efforts were made to obtain separate interviews for each informant. This was generally successful - parents and children were on the whole happy to listen to each other without intervening. However the presence of family members in the room during the interview is highly likely to influence the extent to which important symptoms are freely discussed.

Audio tape recordings and written notes were taken during the interview for later coding and scoring. Information obtained via the CAPA was used to derive diagnoses and symptom scores corresponding to DSM-IV nosology. Symptoms were entered into Excel spreadsheets containing DSM-IV algorithms for attention-deficit / hyperactivity disorder, anxiety disorders and phobias, obsessive compulsive disorder, depression and mania, oppositional defiant disorder and conduct disorder. Symptom counts within each algorithm were added to form dimensional scores. A symptom was counted for dimensional scoring if the subject met the intensity criterion for any item (for example, at least one hour in a single day of intrusive depressive mood once within the 3 month assessment period), regardless of whether frequency or duration criteria were met for inclusion of the symptom into a diagnostic algorithm. Substance use, current medication and current therapeutic input were recorded.

3.2.2 Schizotypy Scales

In addition, symptoms from diverse dimensions which were prevalent in the 22q11DS sample but not in controls were combined to create an index (Table 3.1) of schizotypal disruption with items corresponding to ICD-10 criteria (World Health Organization, 1992). Each item from this list was scored as either present or absent on the basis of symptoms reported in at least one of the parallel questions identified as addressing the same issue within the CAPA. Thus every subject received a schizotypy score ranging from 0 to 9. A positive schizotypy score for each criterion was obtained if the quality of the symptoms reported was deemed to conform to the definition provided by the ICD-10 criteria, even if the symptoms did not meet full criteria for entry into a diagnostic algorithm. This is a highly subjective decision process therefore the validity of these ratings is questionable.

The validity of the schizotypy ratings was investigated by obtaining an additional self-report measure of schizotypal personality using a questionnaire designed for use with

14-17 year olds, the Junior Schizotypal Schedule (JSS), developed by DiDuca and Joseph (1999). The JSS includes 50 questions (answered yes or no). Published factor analysis has indicated that the 50-item form of the JSS has a reliable 5-factor structure, comprising cognitive, perceptual, social, impulsive and physical anhedonia scales. The JSS was completed after cognitive testing by subjects in both 22q11DS and control groups whose literacy levels were good enough to read the questions unaided. The investigator provided explanation of questions when this was sought. The questionnaire was completed by 18 cases and 18 controls.

3.2.3 Premorbid Adjustment Scale

Lastly, a simple index of "premorbid" adjustment commonly used in schizophrenia research (Cannon-Spoor, Potkin, & Wyatt, 1982), was derived from background information on social and scholastic functioning was gathered within the introductory sections of the CAPA (see Table 3.2). Scoring is on a scale from 1 to 7 for each of the five items, according to the descriptive anchor points in Cannon et al (1997). This scale has been used extensively for retrospective assessment of the developmental function of adult patiens, where ratings are usually obtained for two time points (e.g. ages 5-11 and 11-18). In this study the same factors were assessed in terms of present state. The scholastic performance factor (What sort of student is X? Does X come at the top or bottom of his/her class?) was excluded because of the different educational provision for young people with learning disability within both the case and control samples which rendered the institutional experience of school and individual success within the school environment highly variable.

Table 3.1 Derivation of schizotypy ratings from CAPA items

ICD-10 Schizotypal Disorder items	CAPA section	Question codes
1. inappropriate or constricted affect (the individual appears cold and aloof);	Peer relationships	PAM0I01 / CAM0I01 (difficulty making or keeping friends)
	Depression	CDA0I01 (depressed, angry or altered mood)
2. behavior or appearance that is odd, eccentric, or peculiar;	Psychosis	PJB0I01 (idiosyncratic behaviour)
	Observational items	CQA4X01 – CQA4X10 (odd behaviour during interview)
3. poor rapport with others and a tendency to social withdrawal;	Peer relationships	PAM5I01 / CAM5I01 (schizoid lack of interest in people)
	Anxiety	PCA6I01 (social anxiety)
4. odd beliefs or magical thinking, influencing behaviour and inconsistent with subcultural norms;	Psychosis	PJA4I01 (unusual ideas of beliefs)
5. suspiciousness or paranoid ideas;	Psychosis	PJA4I01 (thinking people are against him / her), CJD4I01 (delusions of reference) CJD5I01 (delusions of persecution)
6. obsessive ruminations without inner resistance, often with dysmorphophobic, sexual or aggressive contents;	Obsessions, ruminations and compulsions	CCD2I01 / CCD3I01 and PCD2I01 / PCDI01 (ruminations and obsessive thoughts)
7. unusual perceptual experiences including somatosensory (bodily) or other illusions, depersonalization or derealization;	Psychosis	CJAOI01 / CJA1I01 (depersonalisaton, derealisation)
8. vague, circumstantial, metaphorical, overelaborate, or stereotyped thinking, manifested by odd speech or in other ways, without gross incoherence;	Observational items	CQA5X01 – CQA6X05 (form or speech and social use of language)
	Psychosis	PJA3I01 (psychotic abnormalities of thought and speech)
9. occasional transient quasi-psychotic episodes with intense illusions, auditory or other hallucinations, and delusion-like ideas, usually occurring without external provocation.	Psychosis	PJA2I01 / CJA2I01 and follow-on questions (perceptual disorders and hallucinations
	Psychosis	PJA4I01 / CJA4I01 and follow-on questions (delusions and delusional interpretations

Note: **question codes beginning "P" are derived from parent interview, question codes beginning "C" are derived from child interview.**

Table 3.2 Premorbid Adjustment Scale

Item	Questions	Score	Example
1. Sociability and isolation	Would you describe X as outgoing and liking the company of others or as shy and withdrawn?	1	Not withdrawn, active social interaction
		3	Mild withdrawal, enjoys socialisation when involved—occasionally seeks opportunities to socialise
		5	Moderately withdrawn, given to daydreaming and excessive fantasy, does not seek contact
		7	Unrelated to others, isolated, avoids contact.
2. Peer relations	Does X make friends easily? How many friends does X have? Are there any really close friends?	1	Many friends, close relationships
		3	Casual friends only
		5	Deviant friendship patterns: only friendly with children older or younger
		7	Socially isolated, not even superficial relationships
3. Adaptation to school	Does X get into trouble at school? How much and what kind of trouble?	1	Good adaptation, enjoys school, no discipline problems
		3	Fair adaptation, occasional discipline problems, not very interested in school
		5	Poor adaptation, dislikes school, frequent truancy and discipline problems
		7	Refuses to have anything to do with school—delinquency or vandalism directed against school
4. Interests	Does X have many interests and hobbies? Do his/her interests involve others?	1	Active, involved in a range of school, sporting, and social activities and hobbies
		3	Involved in one school, sporting, or social activity with other young people
		5	Introverted interests—one or a few hobbies which required no contacts with others
		7	No interests—withdrawn and indifferent toward interests of the average youngster.

3.3 *Results*

3.3.1 Case-control differences: rates of psychiatric disorder

As predicted, subjects with 22q11DS displayed higher rates of psychiatric illness than controls, with 79% of individuals fulfilling criteria for one or more diagnoses (Table 3.3). This rate is slightly higher than the 60% rate reported by Arnold et al (2001). Other reports of paediatric psychopathology in 22q11DS have not given a summary figure for rate of diagnoses. The risk of obtaining any psychiatric diagnosis for 22q11DS subjects relative to risk for control subjects in the current samples was 14.4 (95% confidence interval, 3.5 – 58.2).

21% of control subjects met criteria for one or more psychiatric disorders. This is a rather low prevalence rate but broadly in line with other studies of psychopathology in children and adolescents from the general population with learning disability. (Emerson, 2003) reported that 39% of children with intellectual disability met criteria for any psychiatric disorder as compared to 8% of children without intellectual disability. These prevalence rates were obtained via investigation of 264 subjects with intellectual disability (ID) ascertained following a 1999 U.K. Office of National Statistics survey (Melzer, Gatward, Goodman, & Ford, 2000) and assessed using a combined interview and questionnaire method. The criteria for inclusion in the ID group in this study was either recognition by both teachers and parents of learning difficulties or attendance of a special school for learning disability. Hence children with a wide range of difficulties were included, including children with severe impairments.

The rate of diagnosis for controls in the present study is therefore intermediate between that reported for ID and non-ID individuals, although this rate is dependent on a very small number of individuals displaying any suprathreshold disorder (5). In addition it is hypothesised that there were two major self-selection biases in the control sample. One self-selection bias may be a tendency of young people with learning and behavioural problems to be unwilling to take part in research because of a feeling of being selected out as "different". The other self-selection bias would be a tendency of parents of young people with learning and behavioural problems to be additionally willing to take part in research in the hope that their child will directly

benefit from participation. This latter bias may have been encouraged by the provision of brief feedback reports on assessment sessions. Overall, it is hoped that these two biases cancelled each other out to some extent.

The finding of very much increased rates of diagnoses in 22q11DS subjects relative to the control group stands in contrast to the finding of Feinstein et al (2002) of no group differences between VCFS and an IQ matched control group, and the finding of Arnold et al (2001) of elevated mood disorders only. The absolute rates of diagnoses are broadly similar between these three studies (Table 3.4), indicating that any small case-control study is highly susceptible to ascertainment bias and should be interpreted with great caution. There are several possible reasons for the contrasts between the results of these three investigations, the most likely being different ascertainment biases with respect to the control groups. The predominant emphasis of the recruitment information provided for potential controls in the current study was the cognitive and not the psychiatric aspects of the investigation, and this may not have been the case for the previous studies. There is no definitive comparison group against which to obtain a true estimate of the relative risk of psychiatric disorders in a specific population. Use of a comparison group with equivalent levels of psychiatric morbidity might be expected to highlight contrasting profiles of diagnoses, although Feinstein et al did not find this to be the case. The narrower age range of subjects in the current study, and large differences between groups in psychiatric symptoms, may reflect an actual divergence between 22q11DS and other individuals with learning disability during adolescence. Different methods of assessment, namely use of an interview-based instrument rather than a respondent-based computerised assessment (as used by Feinstein et al) could also account for the between-study discrepancies to some degree. Combining interviews from multiple informants, including most importantly the young person themselves, should have increased the validity of the results obtained in the current study. Small sample size hinders the interpretation of all three studies.

A total summary score was computed as the Z-score of the sum of symptom counts. The distributions of this score for 22q11DS and controls are displayed in Figure 3.1. This indicates that levels of symptomatology are normally distributed within the 22q11DS population (Shapiro-Wilk statistic = 0.96, p=0.5) whereas for controls

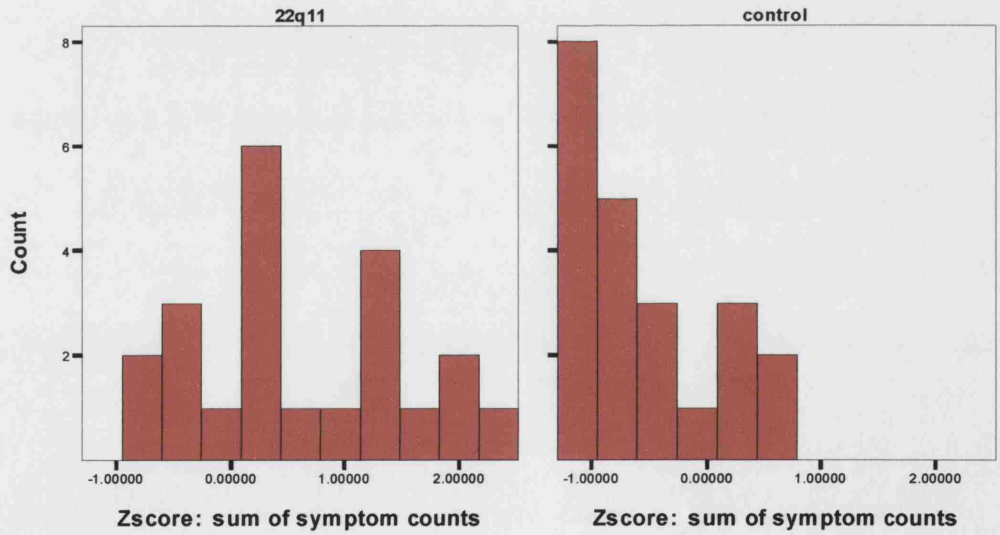
scores are highly skewed towards low levels of symptomatology (Shapiro-Wilk statistic = 0.90, p=005).

Table 3.3 Rates of psychiatric disorders in 22q11DS and control groups

		Group		Chi-square / Kendall's tau-b
		22q11DS (n=24)	**Controls (n=24)**	
Number of subjects meeting criteria for at least 1 diagnosis		19	5	16.3 **
Number of diagnoses for each subject	0	5	19	5.4 **
	1	9	4	
	2	8	1	
	3	2	0	

*** p<0.05 ** p<0.01**

Figure 3.1 CAPA total symptom counts in 22q11DS and control groups



3.3.2 Case-control differences: diagnoses and dimensions

Table 3.4 displays the number of specific diagnoses assigned to members of the 22q11DS and control groups, and symptom counts for each dimension investigated. Odds ratios for the risk of obtaining a diagnosis given membership of the 22q11DS group were similar for all diagnostic categories: 2.6 for any mood disorder (CI 1.7 –

3.9), 2.1 for OCD (CI 1.5 – 2.8), 2.2 for ADD (CI 1.2 – 3.9) and 2.1 for any anxiety disorder (CI 1.3 – 3.4). Comorbidity did not appear to account for these similar risk rates – there was no significant association between the presence of either a mood disorder and an anxiety disorder (confidence interval of odds ratio includes 1), nor was the presence of ADD associated with increased risk of any other disorder. Symptom counts indicated group differences in all dimensions assessed, except for oppositional defiance / conduct problems.

Table 3.4 Rates of diagnoses and symptoms in 22q11DS and control groups

Diagnosis	Number of diagnoses		Chi-square	Dimensional symptom counts (mean, s.d.)		Mann-Whitney U
	22q11DS	Controls		22q11DS	Controls	
ADHD, inattentive subtype	12	4	6.8 *	6.6 (3.1)	3.4 (3.2)	-3.1 **
Any anxiety disorder	10	2	6.7*	1.2 (2.0)	0.2 (0.9)	-2.8 *
Separation anxiety	0	0				
Social anxiety	0	1				
Overanxious	8	1				
Phobia	2	0				
Any mood disorder	9	0	11.1 **	2.7 (2.4)	0.3 (0.9)	-4.4 **
Dysthymic disorder	4	0				
Major depressive disorder	3	0				
Hypomania	2	0				
Manic episode	0	0				
Obsessive compulsive disorder	2	0	2.0	0.7 (1.5)	0.1 (0.4)	-2.0 *
Oppositional defiant disorder / conduct disorder	0	0	0	0.1 (0.4)	0.3 (0.6)	-1.4

*** p<0.05 ** p<0.01**

3.3.3 Comparison with previous studies

Table 3.5 summarises the results of this study in comparison with the two previous case-control studies of psychiatric disorders in children and adolescents with 22q11DS. The profile of psychopathology presented here contrasts with those of the two previous studies in the detection of a high rate of anxiety disorders (not found by Arnold et al) and the low rate of oppositional defiance (in contrast to the findings of both Arnold and Feinstein).

The first inconsistency may reflect either sample differences (age may be an important factor for change in displaying worries, fears and anxieties) or differences between instruments in the threshold of required intensity for anxiety-related disruption required for diagnostic criteria. The second inconsistency - no ODD in our sample - may relate to discrepancies between investigators in the coding of specific symptoms. A marked feature reported for a proportion of 22q11DS subjects in the current study was frequent, brief (generally less than 10 minutes) outbursts of anger. These episodes occurred at least several times a week, and often several times a day, in 9 of the 24 22q11DS subjects. The outbursts do not generally include physical aggression against property or other persons, although in two cases there had been some degree of physical aggression against the self, in the form of banging one's head against walls or hitting oneself. According to both parents and young people, these "rages" are rarely provoked by a clear trigger in the young persons' environment such as not getting one's way or arguments with siblings. The description of these experiences, given by the young people themselves and parents, generally included feelings of being wound-up and frustrated and of feeling unhappy both before and after expressing anger.

Although standardised psychiatric instruments aim to achieve consistency between investigators in the type and severity of phenomena that are coded under a symptom heading, there is still scope for individual interpretation both by the respondent and the investigator. Angry outbursts could arguably be coded in either the ODD (as temper tantrums) or depression sections. There is actual overlap in the questions from the CAPA schedule entered into the ODD and depression algorithms (touchy / easily annoyed and angry / resentful). Since no other ODD symptoms were reported for 22q11DS subjects (for example rule-breaking, disobedience, spiteful behaviour, swearing), whilst other depressive symptoms were frequently reported, it was

decided to code these items within the depression section (double coding within ODD as well is not permitted). Previous studies have reported high rates of ODD, which may reflect either actual differences in behaviours between samples, or different coding of the same "angry" phenomena. This prevalent symptom, which is highly distressing and disruptive for both sufferers and their families, deserves further psychiatric and neurobiological investigation.

Table 3.5 Comparison with previous studies of psychopathology in 22q11DS

	Arnold et al (2001)	Feinstein et al (2002)	This study
Number of subjects	20	28	24
Age range	6-20	6-19	13-23
Control group	Siblings	IQ-matched	IQ-matched
% Generalised anxiety disorder	5 (plus sub-threshold symptoms comorbid with other disorders)	29 (plus 60% phobias and 21% separation anxiety disorder)	33
% ADHD	35	46	50
% Mood disorder	40	29	38
% OCD	15 (sub-threshold symptoms only)	11	8
% ODD	25	43	0

3.3.4 Psychosis-like phenomena

12 subjects from the 22q11DS group (48%) gave coherent reports of psychosis-like phenomena. This was an unexpectedly high rate – Arnold et al reported the presence of "schizotypal traits" (type unspecified) in only 15% of their sample, whilst Feinstein et al reported evidence for hallucinations or delusions in 14%. The most likely reasons for the discrepancies with the findings presented here and previous studies are older age of sample and direct interviewing of subjects. In all but 4 cases where psychosis-like phenomena were reported, parents were unaware of the experiences. In additional cases, parents were aware of some "odd behaviour" such as their son or daughter talking to themselves, having a strong paranormal or religious belief not shared with friends or family members, or being prone to acting out fantasies, without attributing these behaviours to any abnormal mental experience.

It should of course be noted that the task of distinguishing between acceptable but atypical behaviours or beliefs and "symptoms" is difficult and may be conceptually false. For example an exaggerated sense of ones own abilities and future career possibilities or the false perception of being bullied by other youngsters can be seen as either wishful thinking and social distrust, or delusional thinking. Similarly the line between vivid daydreams or memories and hallucinations may be quite fine, although the strength of conviction regarding the external reality of false perceptions should make this categorisation relatively easy. Some of the experiences or beliefs considered "psychosis-like" reported by 22q11DS subjects were disruptive to activity, distressing or frightening for the individual, whilst in other cases the experiences were interesting, comforting or just accepted as "the norm". Subjects with greater degree of learning difficulties may have found the psychosis questions confusing and difficult to understand, but if that were the case it would be more likely for phenomena to be under-reported than exaggerated or invented. It was impossible to get detailed accounts from subjects regarding the onset, frequency and duration of phenomena. Therefore psychosis-like phenomena were considered to be present if they could be classified according to CAPA glossary definitions, regardless of the number of different experiences reported and their perceived severity. Some examples of the reported psychosis-like phenomena are listed in Table 3.6.

Table 3.6 Psychosis-like experiences reported by 22q11DS subjects

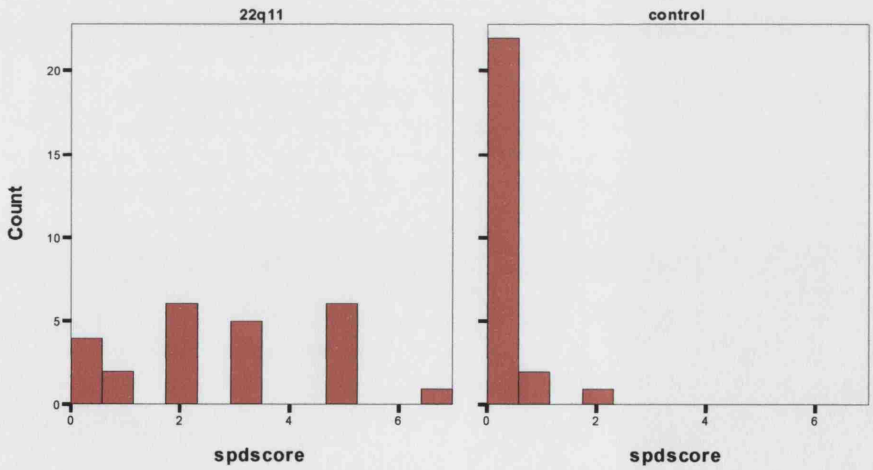
Type of psychosis-like experience		Number of individuals reporting experiences of this type	Examples
Sensory changes and hallucinations	Auditory	10	I hear voices of friends talking or whispering, saying nasty things about me and trying to control me.
			I hear the voice of God speaking from the bible that I like to read at night.
			I hear footsteps on the stairs at night and go out to check who's there. I hear music in my ears that I can't turn off.
			I hear my son talking to objects in his room – it's like he's having conversations with them.
	Visual	5	I saw a woman in old-fashioned clothes coming out of the ceiling in my room. She really frightened me.
			I see my dead granddad and my dead dog in the room with me. Once I saw my friend in the wardrobe when I opened the door.
			She sees her favourite popstars in the room and gets really excited and happy. She gets upset if we tell her they are not really there.
	Other modality	1	Sometimes I smell something horrible, like burning or sewage, and its all around. Sometimes I have a bitter taste in my mouth that won't go away even if I brush my teeth.
Delusions and delusional interpretations		11	I avoid places with CCTV cameras because they let the police know you and then you can get into trouble.
			I feel like there's someone on my shoulder, following me and watching over me. Sometimes this is scary.
			He seems to think that we are all against him, and are trying to stop him from doing things and being happy on purpose.
			He believes his eyes have been damaged or poisoned, maybe by his mother.
			Small things seem really important or really frightening to him, in a way we just can't understand.
Abnormalities of the experience of thought		4	I'm psychic. People can read my thoughts and I can read theirs sometimes.
			There are people, maybe in the clouds or underground, who make us think things and say things. I think they have some kind of a storyboard and can control what the characters do.
			I sometimes feel like I'm an alien from the 24th century and I can see the future and know what is going to happen next.

3.3.5 Schizotypy scores

Distributions of schizotypy scores in both groups are presented in figure 3.2. All but 4 subjects from the 22q11DS group (16%) received at least one positive rating on the schizotypy scale, whilst only 3 control subjects (13%) received any positive ratings. Ratings within the 22q11DS group did not deviate significantly from the normal distribution (Shapiro Wilk statistic = 0.92, p=0.06) although more subjects are required to determine the true distribution of these phenomena.

To assess the validity of the schizotypy scale derived from CAPA items, correlations with the JSS interview measure were assessed. There was a reasonable association between scores for both the combined case and control populations (Spearman's rho = 0.34, p=0.052) and a very good degree of association for the 22q11DS group alone (Spearman's rho = 0.66, p=0.004). The schizotypy scale may therefore have some validity as a measure of the schizotypy construct as used by other investigators. It will be used as a phenotypic indicator within the 22q11DS group (the JSS was not completed by study participants with low literacy levels).

Figure 3.2 Distribution of schizotypy scores in 22q11DS and control groups



3.3.6 Premorbid Adjustment scores

Information from the introductory section of the CAPA concerning family relationships, peer relationships, school performance and spare time activities was used to quantify adjustment according the PMA scales. Data is displayed in Table 3.7. Maximum score for each scale is 7 and for summary total is 28. High scores represent poor adjustment. Levels of "premorbid" adjustment in 22q11DS were markedly below those of controls, on all scales except scholastic adaptation. This dissociation suggests that behavioural disruption consequential to symptoms in 22q11DS is driven to a greater extent by intrinsic factors than by a reaction to the social environment.

Table 3.7 Premorbid adjustment in 22q11DS and control groups

PMA scales	Group - mean (s.d.)		*Mann-Whitney U*
	22q11DS	**Controls**	
Total score	11.9 (3.7)	7.3 (2.2)	-4.4**
Sociability / isolation	3.5 (1.2)	1.6 (1.1)	-4.5 **
Peer relations	3.5 (1.4)	1.9 (0.8)	-4.1 **
Adaptation to school	1.8 (1.1)	1.9 (1.3)	-0.4
Interests	3.2 (1.4)	1.9 (0.9)	-3.4**

***p<0.05 ** p<0.01**

3.3.6.1 Social adjustment

Many (although not all) 22q11DS subjects who took part in this study reported extremely impoverished social interactions and peer relationships. Many have no friends (although there was often a degree of inter-informant disagreement on this factor, with parents reporting no friends at all, and their child reporting that they had lots and lots of friends although they were usually unable to name one when asked). Most had very little informal contact with peers outside school. Relative to controls, they also participated far less frequently in organised social activities such as youth clubs or hobbies. There were a couple of exceptions here, for example one subject was an enthusiastic member of a drama group, another of a golf club and another of a local youth club for teenagers with learning disability. However it was notable that in all

three of these cases, interactions with young people within the context of a group activity had not led to the formation of peer relationships that extended outside of this activity.

Individual reactions to, and explanations for, the lack of social contact (provided by both parents and young people) varied considerably. Some of the subjects appeared to show little interest in young people of their own age, preferring either the company of much younger or older individuals, or seeming genuinely content with their own company and solitary pastimes. A contrasting response demonstrated by a significant proportion of subjects was to feel extremely lonely and unhappy as a consequence of lack of friendships and social activities.

In terms of explanations for social isolation, a proportion of the group had experienced bullying or rejection by peers (or perceived that they had been the recipient of these negative experiences) and had thus lost confidence with and trust in their peers. Many parents reported that their son or daughter did not demonstrate mature social skills or social understanding on a par with their peers, sometimes (but not always) in combination with a lack of confidence and high levels of anxiety in social situations. Others reported that their son or daughter seemed to lack an ability to form close bonds to others (sometimes including family members), either forming casual and fleeting alliances with many people, or appearing aloof and disinterested. Some subjects showed an apparent intolerance for the wishes or needs of other young people, which either caused the 22q11DS youngster to withdraw from friendships, or potential friends to shy away from these often quite demanding individuals. Others were causing concern to their parents by choosing apparently unsuitable friends, either because of age-differences or because of a fear that their son or daughter may be easily led or taken advantage of (although this may be a common feeling amongst parents of teenagers, particularly those with learning disability).

3.3.6.2Interests

The spare time activities enjoyed by individuals with 22q11DS were often impoverished or unusual. It is important to note that there was wide variation in the opportunities provided to each individual for supported social contact with young people of equivalent levels of general cognitive and communication ability, and for

participation in hobbies and activities. However even when many such activities were on offer, the solitary option was often preferred. Many subjects were reliant on activities such as TV, listening to music and computer games for occupation, requiring little self-motivation or active participation. However these activities were also very prevalent in the control population, particularly when local amenities were poor or required considerable travel, and parental encouragement to try more active pursuits was somewhat lacking. There were, on the other hand, three types of activities that were reported only by 22q11DS subjects, which may be more informative of the relationship between the syndrome and other developmental disorders, and also of the underlying nature of distinct psychopathology.

Firstly, some subjects were reported to have extremely strong, almost obsessive interests, in a single highly defined activity or topic, for example following a particular pop group, wanting to go to a particular location or shop, or in one case taking a very strong interest in weather patterns. A striking feature of these interests, which are not in themselves at all unusual, would be that they would begin very suddenly and end just as abruptly, whereas in the interim they are almost totally absorbing. Both the abrupt changes and the obsessional interest were reported to be difficult to comprehend and disruptive to family life. Whilst this type of absorption is again quite reminiscent of autism, these interests were not associated with highly repetitive, stereotyped behaviours. One interpretation of these behaviours is that for a period of time these topics acquire "special significance" for the individual concerned.

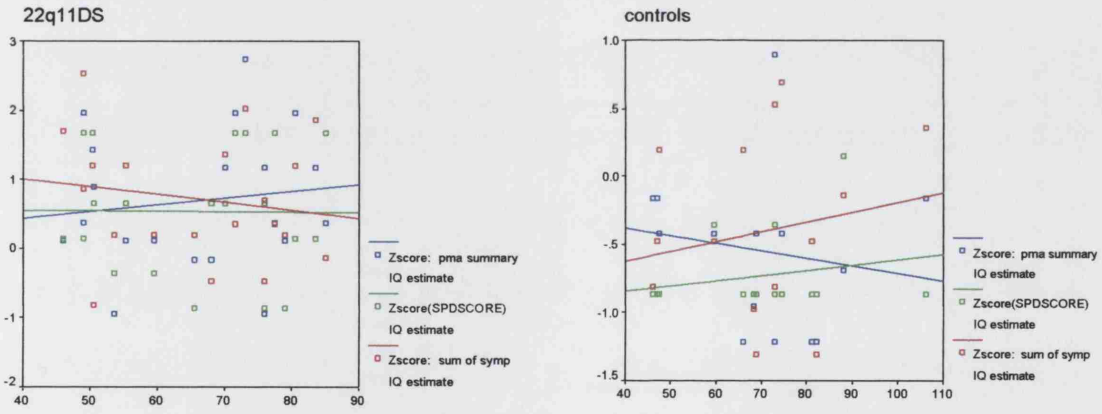
A second unusual feature was parental report of fantasy play or role-playing activities. There was a range of behaviours described within this category, from singing-along and dancing to pop music and pretending to be the pop-star in question, to playing with dolls and action men, to sitting in the family car for many hours at a time, employed in some fantasy activity that could not be precisely specified by the parent in question. Although these activities may be quite normal in younger children, they are unusual in late adolescence (at least away from the context of karaoke bars and science fiction clubs!). These phenomena may indicate a tendency for schizotypal blurring of the boundary between fantasy and reality (also evidenced by some rather unrealistic expectations of future career prospects, for

example football star, radio D.J., RAF pilot). However this interpretation is provided very cautiously given that it is a rather obvious conclusion to jump to in individuals known to be at high-risk for psychosis. Further investigation of these developmental cognitive biases (if that is what they are) should be investigated in more detail and, if possible, blind to genetic diagnosis.

3.3.7 Relationships between psychopathology and IQ

Neither the summary score for CAPA symptoms, nor the schizotypy score, nor the total PMA score were correlated with estimated full-scale IQ for either the 22q11DS group or controls (Figure 3.3). This indicates that psychiatric symptoms and functional impairments are not reflections of non-specific cognitive impairment in this sample.

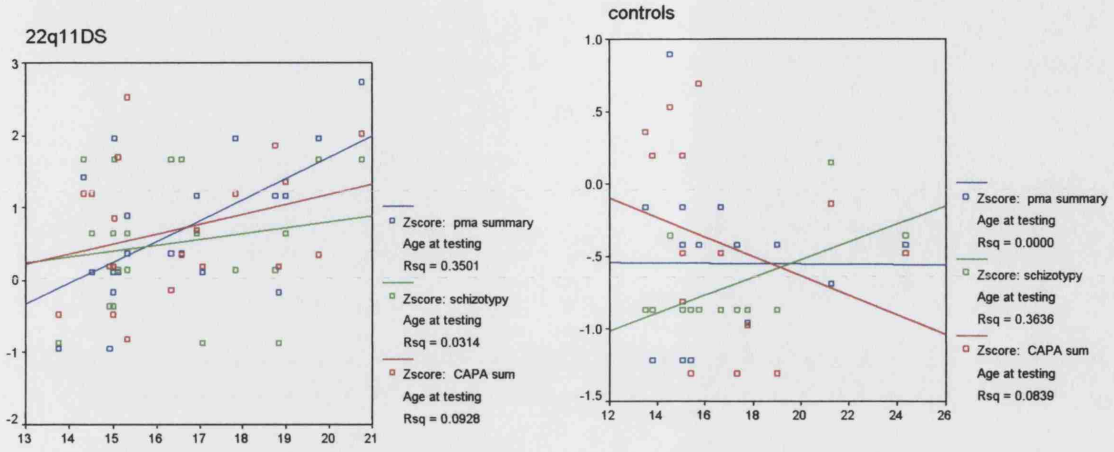
Figure 3.3 Psychiatric symptoms and IQ in 22q11DS and control groups



3.3.8 Relationships between psychopathology and age

The only relationship between psychopathology and age found for either group was an apparent increase in maladjustment in older 22q11DS subjects (Spearman's rho = 0.41, p=0.046). This suggests that although psychiatric and schizotypal symptoms do not seem to arise as a consequence of adolescent development in the 22q11DS group, the social and functional impairments associated with these abnormalities increase, either absolutely or in comparison with peers and expectations of parents.

Figure 3.4 Psychiatric symptoms and age in 22q11DS and control groups



3.3.9 Inter-relationships between CAPA summary score, schizotypy and PMA ratings

Partial correlations were computed for the three summary measures of psychopathology, controlling for age on the basis of the association with PMA scores in the 22q11DS group. This analysis was only justified for the 22q11DS group because of the limited variance of scores in the control group. This indicated a highly significant relationship between schizotypy scores and PMA ($r=0.70$, $p=0.001$) but only a trend towards a relationship between CAPA summary score and PMA ($r=0.35$, $p=0.14$). Schizotypy scores and CAPA summary scores were also unrelated to each other ($r=0.12$, $p=0.6$). This suggests that schizotypy, comprising social and emotional disruption and psychosis-like phenomena, is an independent dimension of symptomatology in 22q11DS, which makes a specific contribution to day-to-day functioning.

3.4 Discussion

3.4.1 Summary

- Adolescents and young adults with 22q11DS display diverse psychiatric symptoms, with high rates of almost all diagnoses examined. These rates were much higher than those seen for controls, but, more importantly, they were broadly in line with previous reports of psychopathology in children and adolescents with 22q11DS.

- The most prevalent psychiatric symptoms in the 22q11DS group were mood disruptions, attention deficits and psychosis-like phenomena.
- The majority of individuals with 22q11DS displayed at least some symptoms of schizotypal disorder, according to interview-based assessment and using ICD-10 criteria.
- Psychiatric symptoms were independent of IQ in both 22q11DS and control groups, and are therefore unlikely to be a non-specific consequence of learning disability.
- Within the 22q11DS group, premorbid adjustment scores were higher in older subjects, suggesting increasing functional impairments with age. However, rates of psychiatric symptoms, including schizotypy, did not increase with age.
- More severe schizotypal disruption in 22q11DS was associated with poorer social functioning and fewer interests, as measured by the PMA scales.

3.4.2 Limitations

- Small sample size and self-referral biases mean that results presented here may be either over-representative or under-representative of the true rates of psychopathology in both 22q11DS and control populations.
- There is no "perfect" comparison group for a psychopathological study, since there can be no definition of a normal personality. However, comparison to more than one additional group would clarify the specificity of features to 22q11DS, and determine continuity with other disorders sharing either psychiatric or neurocognitive features. Populations that could be compared with 22q11DS in future studies to extend these findings are early-onset psychosis, a clinic sample of non-psychotic adolescents with psychiatric disorders, high-risk individuals (relatives of patients), high-risk individuals (showing prodromal symptoms) and individuals with other genetic syndromes.
- All interview or questionnaire-based studies are hampered by the limits of self-report. Some respondents (both young people and parents) may not have understood questions, or comprehended them in a manner different from that intended by the instrument. This is a particular difficulty in learning disability.
- The corollary of this limitation, no less likely to introduce error, is the failure to understand, or misinterpretation of responses, by the investigator. This may be particularly true for respondents with communication impairments, or where

there is marked difference between the cultural and social context of the respondent and the interviewer.

- All interviewing, coding and scoring of material was conducted by a single investigator in this study, so it is highly likely that her preconceptions, judgements and inconsistency rendered some results unreliable. To combat against this, case summaries were discussed and extracts from audio-tapes reviewed with two senior child and adolescent psychiatrists, both when the investigator had some doubts about correct coding and when she did not. However in future blind double-coding should be carried out to establish validity and reliability.
- The measures used in this investigation had several limitations. Firstly, no specialised interview was conducted to establish schizotypal features, but a post-hoc scale constructed on the basis of CAPA items. Secondly, the CAPA is not a truly dimensional instrument designed to measure individual differences in psychological well-being. It is intended first and foremost to establish the presence or absence of psychiatric diagnoses which, whilst highly appropriate in some circumstance, was not the primary aim of this investigation.
- A particular problem ensues when using a categorical instrument to determine levels of psychopathology in a developmental disorder. CAPA, like other instruments of its type, requires information about the onset of symptoms for each criterion, but in a developmental disorder it may be nonsensical to enquire as to when a symptom occurred. For example, if 22q11DS is associated with developmental dysregulation of mood, such that some children with the syndrome have always been unhappy or overexcited most of the time, it is impossible to establish an "onset" of symptoms, even at current severity or current level of social unacceptability. Similarly, symptoms may fluctuate, such that no individual episode meets criteria for frequency and duration of symptoms, but long-term developmental disruption is still severe. Lastly, individuals with a neurodevelopmental disorder are likely to display symptoms that cut across many different categories, so that the diagnostic instrument may artificially generate many comorbid diagnoses not representing the true mental health of the individual, or may result in many subthreshold scores across several categories, under-representing actual morbidity.
- CAPA does not enquire about symptoms contributing to autistic spectrum disorders. Given the potential overlap between symptoms of a psychotic disorder

and autism, and previous reports of autistic features in 22q11DS, this limits the comprehensive cover of this assessment.

- Two sections of the CAPA were excluded from this assessment. The lengthy eating disorders schedule was omitted to save time and because of the lack of previous evidence suggesting that this domain was problematic in 22q11DS. The life events section was also omitted, because the interaction between symptoms and environmental events is likely to be complex and was not the primary focus of this investigation. A future study should move beyond the elucidation of symptoms to this important question.

3.4.3 Implications - The spectrum of psychopathology in adolescents with 22q11DS

3.4.3.1 Continuity and discontinuity of psychopathology in 22q11DS with disorders in the general population

On the basis of data collected here, no clear subgroup of 22q11DS subjects emerged demonstrating either markedly worse overall psychiatric and behavioural function, or much greater tendency towards schizotypal features. For all dimensional measures, histograms did not point towards bimodal distributions. This suggests that there is a continuum of psychiatric disorder within the 22q11DS group, from mild to severe, with no junction between affected and unaffected, at least during adolescence. However it is important to provide two caveats to this observation. The presence or absence of bimodality cannot be conclusively determined in a sample size this small, and sampling bias may have meant that one or other tail of the distribution could be over-represented. Another argument against a distinct, psychosis-prone subgroup, is the lack of association between different symptoms. However, in a larger sample and using more appropriate measures, distinct dimensions of psychopathology which are expressed independently of each other and cluster together within 22q11DS individuals may be identified.

The presence or absence of psychosis-like phenomena could be argued to represent a distinct division within the 22q11DS group, which may be relevant to risk of later illness. However this is again unlikely, given the difficulty of defining a borderline between, for example, "heightened normal suspiciousness" and "mild paranoia".

Even hallucinations are difficult to differentiate from vivid daydreams, especially when the conviction regarding their basis in reality is diminished at the time of interview, which may be days or even months after the experience. Whilst it seems intuitively plausible that the more intense, frequent and prolonged the psychotic experiences, the more likely the evolution of illness, temporal fluctuations in phenomena may mean that a single "snapshot" of these elements of psychopathology will not accurately predict long-term outcome.

Continuity between the symptoms and experiences of the young people studied here and the manifestations of idiopathic psychosis can only be speculated. The features reported correspond to the broad outline predicted for a schizophrenia-prone population, but in some individuals affective symptoms were dominant, in others anxieties and obsessions very problematic. This may mean that psychopathology in 22q11DS is continuous with psychiatric disorder experienced by the general population (a) in an entirely non-specific fashion with all diagnoses being prevalent in 22q11DS, or (b) only with respect to disorders that feature psychosis, or (c) with schizophrenia in particular. Only a comparison study using this same method to assess psychiatric symptoms and adjustment in equivalently-aged patients with either psychosis or non-psychotic disorders will provide evidence for one or other conclusion. It would be especially informative to compare the 22q11DS group with high-risk adolescents (for example siblings of patients), and with patients who have experienced but "recovered" from an acute episode of psychosis, since it is predicted that the diversity of symptoms in these two populations may match that of 22q11DS individuals.

In some respects, lack of social skill and impoverished social experience in 22q11DS (for example intolerance of other's perspectives, lack of social tact and an inability to "fit-in") are similar to autistic spectrum traits. In other respects, for example suspiciousness of peers and social avoidance, the descriptions provided by both parents and young people seemed similar to schizoid characteristics, as defined by Woolf and colleagues (Wolff, Townshend, McGuire, & Weeks, 1991). Another difference between 22q11DS and autistic spectrum characteristics, reported by some parents, was either good or overly acute sensitivity to the emotions of other people, rather than a lack of such awareness.

A possible explanation for this apparent contradiction is that psychosis may be associated with heightened sensitivity and attraction to social cues, but not necessarily correct processing and interpretation of social signals, whereas in autism such stimuli are either uninteresting or aversive. Therefore individuals with autism and with 22q11DS or idiopathic schizophrenia may have similarly impaired social function, but for reasons of contrasting deficits in neural circuits underlying "social cognition". This hypothesis could be tested in 22q11DS in a future study employing both behavioural and neurophysiological techniques to explore social processing.

The judgement of psychotic and emotional symptoms as either eccentricities of personal identity, or problems requiring treatment, differed markedly between the families of 22q11DS subjects, and sometimes within families, often with resultant conflict between family members. In some cases this conflict, and a fear of receiving a daunting diagnosis with the stigma that it entails, was obstructing access of young people to mental health support when they quite seriously required it. This difference in individual conceptualisation of psychosis, and psychiatric symptoms in general, mirrors conflicts within wider society over diversity in personality, and reactions to mental distress in oneself and in others. Anthropological investigation of belief-formation, decision-making and reactive behaviour in families of individuals with 22q11DS could inform debate on this topic, and also provide some practical insights of use to families, and professionals working with 22q11DS and other "high-risk" groups.

Continuity between the atypical experiences reported at relatively high frequency in this population, and non-pathological variation in psychological function and personality in the general population is more difficult to establish. There was very little overlap between the features displayed by the 22q11DS group and the controls assessed in this study, suggesting that the profile is distinctly "abnormal". However, no feature reported by any subject was beyond comprehension as a reasonable human emotion or imaginable perceptual experience or belief. The continuum of experiences and behaviours seen in 22q11DS is in line with work pioneered by van Os and colleagues on the "normal" experience of psychosis-like phenomena for a proportion of the general population. Finally, a continuum from atypical but non-problematic personality features to disruptive and disabling symptoms is predicted within the construct of schizotypy - a dimension of normal personality variance that

may in fact have some advantageous consequences such as creativity and single-mindedness (Gruzelier, 2003).

3.4.3.2 Continuity and discontinuity of psychopathology across the developmental time-span in 22q11DS

Although it is not of course known whether any of the individuals who took part in this study will manifest a psychotic illness (or any other major psychiatric disorder) in future years, there is considerable symptomatology already present in this sample that is reminiscent of such disorders. This suggests that, if such an illness does develop, it will not arrive unannounced but will emerge from a developmental context of atypical, and in many cases highly disruptive, emotions, thoughts and behaviours. The lack of an association between age and either DSM-IV diagnoses or schizotypal features, within this study group, suggests that developmental events prior to adolescence are sufficient to mediate vulnerability to these symptoms. However, the significant relationship between age and poorer premorbid adjustment (which is itself correlated with the intensity of both schizotypal and general psychiatric symptoms) suggests the possibility of functional deterioration during the progression from child to adult. This may signify more intrusive symptoms during later adolescence secondary to increased neurocognitive disruption, or deteriorating social and occupational disruption secondary to increased demands on the individual. It is also very plausible that this effect of age is an artefact of small group numbers. Only longitudinal assessments to determine within-subject change over time, and recruitment of a larger number of subjects will adequately address this question.

3.4.3.3 The relationship between psychopathology and cognitive function in 22q11DS

The fact that such a diverse range of symptoms and behavioural disruptions were reported for 22q11DS subjects suggests two competing hypotheses about the relationship between cognitive and psychiatric disorder within the syndromal group.

A single neurocognitive mechanism may have a very wide-ranging effect on psychological development in 22q11DS individuals, such that the whole function of the personality is vulnerable, with individual symptoms arising in the context of

environmental interactions and the trajectory of emotional-behavioural maturation. The lack of association between symptom-clusters within 22q11DS supports this argument for a rather chaotic relationship between neurocognitive vulnerability and overt expression of symptoms. Alternatively, many different neurocognitive dimensions could be independently vulnerable as a consequence of the microdeletion, with each symptom cluster resulting directly from an impaired system. This hypothesis is supported by the consistency between subjects in the characteristics of certain symptoms, for example angry outbursts and mood swings, which suggests disruption to either mood regulation or contextually-appropriate emotional behaviour. The frequent reporting of psychosis-like phenomena is ambiguous with regard to these two hypotheses. On the one hand, these symptoms may be dependent on one underlying neurocognitive system, for example self-monitoring or inhibitory control. On the other hand, the diversity of experiences (no strong bias towards either abnormal sensory experiences, delusional ideation or thought disorder) argues against one-to-one mapping between neural dysfunction and mental experience.

The relationship between psychiatric symptoms and neurocognitive impairment will be discussed further in the following sections, in which the potential impact of endophenotypic features on dimensions of psychopathology will be explored.

4 Working memory - a potential cognitive endophenotype for psychosis in 22q11DS

4.1 Introduction

4.1.1 Cognitive function in schizophrenia

Impairments in cognitive function are increasingly central to descriptions of both the clinical phenotype and neuropathological underpinning of schizophrenia. However perspectives on the relationship between cognitive deficits and the symptoms of schizophrenia vary. Cognitive disability may arise as a secondary consequence of the devastating psychological disruption of psychosis, of the long-term psychosocial disruption experienced by many sufferers of mental illness, or as side-effects of the powerful neurobiological effects of anti-psychotic medication. This would predict that cognitive impairments emerge over the course of a psychotic illness. This is not the case – cognitive dysfunctions characteristic of schizophrenia are present in first-episode patients (Bilder et al., 2000), including patients with early-onset illness (Kravariti, Morris, Rabe-Hesketh, Murray, & Frangou, 2003). Cognitive impairments are also detectable in high-risk individual prior to the onset of illness (Erlenmeyer-Kimling et al., 2000), although some impairments may worsen over time or be exacerbated by anti-psychotic mediations (Cosway et al., 2000).

The opposing neuropsychiatric viewpoint is that a direct causal relationship exists between neurocognitive disruption and the subjective experience of a psychotic disorder. For example, Frith and colleagues have proposed that an abnormality in self-monitoring of one's actions directly underlie delusions of agency, a theory for which there is both neuropsychological and neuroimaging evidence (Frith et al., 2000). Another cognitive theory for the emergence of psychosis has been proposed by Kapur (2003), who suggests that alterations in the attribution of salience to environmental stimuli predisposes to development of delusional ideation, coherent with neurocognitive models of altered sensory gating (Cadenhead et al., 2000) and cognitive control (Barch, Carter, MacDonald, Braver, & Cohen, 2003). This viewpoint suggests that cognitive deficits will emerge in parallel with symptoms. This hypothesis is difficult to test, but may be possible in the context of ultra high-risk, early identification and prevention studies.

One can also view cognitive dysfunctions as co-morbid phenomena to the psychopathology of schizophrenia. Abnormalities in the development and function of neural circuitry could give rise independently to specific cognitive impairments and psychopathological experiences. In this situation, cognitive impairments could be considered more as indicators of at-risk neurobiology rather than at-risk neuropsychiatric states. Impairment in general intellectual ability and specific cognitive functions such as memory and executive function are usually found to correlate with negative symptoms such as avolition, alogia and altered affect, rather than with positive symptoms (O'Leary et al., 2000). These symptoms tend to be continuous, relatively resistant to anti-psychotic medication, and may precede the onset of psychosis in the form of poor premorbid social and educational adjustment (Fennig, Putnam, Bromet, & Galambos, 1995).

Cognitive impairments and features of the negative syndrome have been detected fairly consistently in family members of individuals with psychosis, indicating that they may be a reflection of genetic susceptibility. Tsuang, Stone and Faraone (2000) have advocated a reformulation of the schizophrenia diagnosis with emphasis placed on the cognitive and negative components (rather than on the end-point of psychosis). They conceptualise the core disorder as "schizotaxia", a term first used by Meehl to describe a familial and neurodevelopmental at-risk state for psychosis. In this model, psychosis is an unpredictable precipitate of a vulnerable neurodevelopmental system in combination with adverse environment and chance, which is not informative regarding the underlying neurobiology and causation of schizotaxia. Tsuang et al have proposed specific criteria for the diagnosis of schizotaxia and have also suggested that it can be treated with low-dose anti-psychotic medication, both to ameliorate cognitive and negative symptoms and to prevent progression to full-blown schizophrenia (Tsuang, Stone, Tarbox, & Faraone, 2002). Although this presents the at-risk cognitive state as a categorical entity, it is equally possible that there is continuous variation within the general population in genetically-influenced cognitive vulnerability that predisposes to both negative and positive symptoms, and that therefore corresponds to the definition of endophenotypes that could be informative for genetic studies.

4.1.2 Identifying potential cognitive endophenotypes in schizophrenia

In chronic schizophrenia, cognitive impairment can be a pervasive and non-specific neurodisability (Tamminga, Buchanan, & Gold, 1998). Individuals with a more severe course to their illness may manifest these impairments as a consequence of more pervasive neurodevelopmental abnormality, or additional neurodegenerative processes, or as a consequence of long-term neuroleptic treatment. Hence they are not the best population in which to detect signs of the cognitive endophenotype associated with genetic risk. However, family studies have indicated that there are a number of neurocognitive traits that can be independently inherited in association with elevated risk for schizophrenia (Tuulio-Henriksson et al., 2002). Over the past few years it has been possible for the first time to conduct studies that identify regions of the genome, and even specific genes of influence, that are associated with these traits.

Probably the most consistent findings from family studies are that deficits in attention, memory and executive functions are found in association with risk for schizophrenia, both in family members and individuals identified as being at-risk as a consequence of displaying features of schizotypal personality. Although there are multiple neuropsychological paradigms that have been used to assess endophenotype-like disruption in at-risk individuals, there is considerable overlap in the neurophysiological processes that such tasks may tap. Most tasks for which there is evidence of endophenotype status involve processing and integration of simple information prior to the selection and execution of some response and inhibition of others. This type of on-line processing, whilst undoubtedly complex and involving multiple overlapping cognitive and neurophysiological systems, can be conceptualised and studied in a relatively simple fashion as the domain of working memory.

4.1.3 Working memory and schizophrenia

Working memory refers to a set of cognitive processes that enable information to be maintained and manipulated for immediate recall and continuous performance of any task. There are several different models of the component processes of working memory, but the most influential is that of Baddeley and Hitch (1974). This states

that the working memory system comprises two storage and rehearsal systems (the visuospatial scratch pad and articulatory-phonological loop), and a central executive for attentional control, multi-modal integration and immediate processing tasks. Working memory is usually tested by providing sequences of information for immediate recall, for example strings of digits, words or spatial locations. By varying the conditions under which the information must be reproduced, for example by requiring the reversal of sequence order on repetition, increasing the delay time between presentation and recall, or presenting distracting information that must be inhibited for task success, the capacity, speed and independence of each sub-system can be examined. Working memory is a whole-brain skill, although its component processes (encoding, rehearsal, retrieval, sequencing, inhibition) are dependent on more localised subsystems; sensory and spatial systems in diverse cortical regions, encoding systems in structures such as the hippocampus, and monitoring / control systems within the frontal lobes and particularly the prefrontal cortex (Baddeley, 1996). The efficiency of the whole process is dependent on adequate functional connectivity between all regions.

Many studies have demonstrated working memory abnormalities in schizophrenia (Keefe, 2000). Both verbal and spatial tasks have been used to demonstrate impairments, indicating no strong dissociation between the integrity of phonological or visuospatial components within patient populations. Group differences on working memory tasks tend to be more pronounced with increased task difficulty, suggesting that impairments are only seen in tasks that require the engagement of the central executive. For example, in the N-back working memory task, a sequence of stimuli are presented (either a single digit or a target stimulus within an array of distractors) and subjects must recall the target either immediately following presentation (0-back condition), or respond according to the preceding one or two stimuli (1-back and 2-back conditions). Deficits in this task are usually seen in schizophrenia patients only during the 2-back condition (Carter et al., 1998), but see Abi-Dargham et al (2002) for a failure to replicate this result. Patients perform poorly on other tasks requiring attention switching and inhibition of prepotent responses (such as the classic Stroop task), and supervisory attention (such as the Continuous Performance Test), suggesting an overriding difficulty in cognitive control (Braver, Barch, & Cohen, 1999) that could impact on working memory in multiple paradigms.

The application of in vivo functional imaging techniques has suggested that deficits in cognitive control and working memory in schizophrenia may arise as a consequence of hypoactivation of the prefrontal cortex, an area engaged during both working memory and attentional control (Carter et al., 1998; Perlstein, Dixit, Carter, Noll, & Cohen, 2003). However since hypoactivation is seen when task difficulty increases and performance declines in both patients and controls (Callicott et al., 1999), a causal connection between diagnosis and prefrontal hypoactivation cannot be established. The claim for hypoactivation is also confounded by the issue of resting or control baseline conditions, which may not be equivalent in patients and controls. An increasing number of studies have demonstrated deficient integration of neural processing in schizophrenia during working memory when task performance is controlled for, using positron emission tomography (Meyer-Lindenberg et al., 2001) and functional MRI (Weinberger et al., 2001). These studies implicate a distributed sub-cortical and cortical network involving the dorsolateral prefrontal cortex, the anterior cingulate, areas of the parietal and temporal lobes and the cerebellar hemispheres. The impact of task performance on the activation of either network in both patients and controls suggests that modulation of circuit activity in different cognitive contexts may be a contributory factor to dysfunction. Thus decrements in working memory performance in schizophrenia, as a consequence of failure to appropriately recruit a circuit involving the prefrontal cortex, may not represent a static "lesion-like" cognitive deficit, but rather a limited ability to regulate a normal, dynamic neurocognitive process.

Because the basic concept of working memory – temporary maintenance of information within neural systems to facilitate further processing – is fairly simple, it is a good model for mapping between different levels of analysis (behavioural, cognitive, neural, cellular and molecular). Candidate cellular mechanisms that could influence the neural underpinnings of working memory function include synaptic plasticity, neurochemical regulation or neural pathway organisation during development. Genetic regulation of these mechanisms provides a plausible set of pathways contributing to individual differences in the development and function of schizophrenia-relevant cognitive systems. Hence the identification of genetic factors that influence working memory, using the endophenotypes approach, would

provide a route towards an increased understanding of the molecular pathophysiology of the illness.

4.1.4 Working memory as a potential endophenotype for schizophrenia

Progress in characterising working memory as an endophenotype for schizophrenia is summarised in Table 4.1. There is considerable evidence that both verbal and non-verbal working memory function is disrupted in schizophrenia (and not in other psychoses), at early stages in the illness, and with continuity in the face of clinical change. There is some evidence that spatial working memory is more specifically and stably affected than performance on verbal tasks, but this may be an artefact of the tasks employed. Spatial span is usually tested by oculomotor or motor delayed response tasks, which may be more pure measures of information maintenance, inhibition and reproduction. Verbal tests, which involve lexical access and may activate semantic items within long-term memory, may be less consistently compromised depending on the task procedure.

Studies of adult relatives and affected-unaffected twins indicate that verbal working memory impairments may be traits associated with genetic liability, perhaps impacting to a greater extent on complex tasks that stress the central executive component or increase integrative demands. High-risk studies have also indicated that working memory abnormalities are present before adulthood in at-risk individuals, and that more severe disruption of this neurocognitive system during development is predictive of poor psychiatric outcome. Taken together, this is evidence that working memory performance reflects genetically influenced continuous variation within the population in the integrity of neurocognitive systems implicated in psychiatric well-being. Working memory dysfunction may be a reflection of necessary neural dysfunctions for schizophrenia-risk, or one of many possible pathways towards vulnerability. There does not appear to be specificity for visuospatial, verbal or central executive working memory deficits as endophenotypic markers. Definition of a cognitive endophenotype more reflective of physiological regulation may determine whether there is one underlying neurocognitive basis for working memory deficits as a risk indicator.

Table 4.1 Evidence in support of working memory as an endophenotype for schizophrenia

Reference	Study population	Summary of findings
CRITERION 1 – ASSOCIATION WITH ILLNESS RISK		
Illness study (first- episode)		
(Bilder et al., 2000)	94 SZ / SZA after initial stabilisation (~6 months after presentation), 36 controls	41 tests collapsed to 6 scales. Greater impairment on memory and executive scales than on language, attention, motor and visuospatial. A high ability subgroup (median split on global scale) showed only memory deficits. Memory scale comprised diverse tests, and performance correlated with other domains.
(Saykin et al., 1994)	37 neuroleptic-naïve first episode SZ, 65 unmedicated previously treated SZ, 131 healthy controls	Patient groups have similar patterns of generalised impairment, with verbal memory and learning accounting for group differences. Spatial cognition, motor speed and visual memory only impaired in treated group.
Test-retest stability in face of clinical change		
(Hoff et al., 1999)		Only verbal memory does not improve relative to controls over a 5 year period of remission from first-episode of psychosis.
Specificity for schizophrenia		
(Park & Holzman, 1992)		BPD cases perform better than controls, whilst SZ performed worse, on an oculomotor delayed response task (an analogue of spatial working memory). Verbal abilities not consistently found to be different between groups (in other studies)
(Keri, Kelemen, Benedek, & Janka, 2001)	Siblings of patients with SZ (25), BPD (20), controls (20) matched for age, IQ and global functioning.	SZ-sibs impaired on visual backward masking and spatial working memory. SZ-sibs and BPD-sibs both impaired on long delay verbal recall test. Indicating some shared and some non-shared genetic / neurocognitive components.
Spectrum study – schizotypal personality disorder		
(Roitman et al., 2000)	29 SPD, 33 other personality disorder, 31 controls	Attention deficits, verbal learning and visuospatial working memory deficits detected in SPD patients only.
(Voglmaier et al., 2000)	16 SPD, 16 normal controls (all male)	Generalised decrement in most tests requiring short term retention of information, more marked for verbal tests.

Reference	Study population	Summary of findings
Spectrum study – psychometrically defined schizotypy		
(Park, Holzman, & Lenzenweger, 1995)	High scoring (28) and low scoring (23) college students on Perceptual Aberration scale.	High scorers impaired on a delayed match spatial working memory task, and not on Wisconsin Card Sorting.
Family study – adult relatives		
(Conklin, Curtis, Katsanis, & Iacono, 2000)	SZ 52, first-degree relatives 56, controls 73	SZ patients show deficits on forward and backward digit span. Relatives differ from controls on backward digit span only.
(Toulopoulou, Morris, Rabe-Hesketh, & Murray, 2003)	SZ 70, first-degree relatives 115, controls 66	Well-relatives impaired relative to controls on immediate verbal recall, verbal memory and strategy formation.
Family study – adult relatives, specificity for schizophrenia		
(Kremen, Faraone, Seidman, Pepple, & Tsuang, 1998)	Female relatives by diagnosis: SZ 39, bipolar disorder 15, no diagnosis 44.	Auditory attention, verbal and visual memory deficits in SZ relatives only
Family study – high risk children		
(Erlenmeyer-Kimling & Cornblatt, 1992)	Two cohorts of offspring with parental diagnoses: SZ 109, Aff 82, no diagnosis 165	Offspring of SZ parents perform more poorly on all measures. Tests include Continuous Performance Test (sustained attention), forward and backward digit span, visual-aural digit span. Five factors derived: error and correct responses on CPT, verbal memory, find motor and gross motor skills.
Prospective study – high risk sample		
(Erlenmeyer-Kimling et al., 2000)	Parental diagnoses: SZ 79 (15% of whom developed SZ by time of follow-up), Aff 57 (7%), no diagnosis 133 (0.8%)	Verbal memory is strongest predictor of schizophrenia-related psychosis in offspring of SZ parents (sensitivity = 83%, false positive rate = 28%).
(Cosway et al., 2000)	78 offspring of SZ parents (19 with psychosis at >2 year follow-up), 22 controls.	Verbal memory deficit discriminates high-risk subjects with and without psychosis from controls. Decline in IQ and verbal memory detected between initial assessment and follow-up for high-risk subjects converting to psychosis.

Reference	Study population	Summary of findings
CRITERION 2 – HERITABILITY		
Twin study (general population)		
(Ando, Ono, & Wright, 2001)	143 MZ and 93 DZ twins	Heritability of each WM test ~ 45%, accounted for by a single general factor (general ability?) with heritability = 65%. Additional, modality specific factors contribute to additional variance. Storage and executive components for verbal and spatial modalities.
Twin study (affected-unaffected)		
(Cannon et al., 2000b)	Twins discordant for SZ: MZ (18), DZ (30). Control twins: MZ (28), DZ (27). Twins concordant for SZ (8).	Spatial working memory, divided attention, choice reaction time and recall intrusions are independently sensitive to genetic loading for SZ suggesting distinct sets of susceptibility genes. Higher prevalence of schizophrenia-related personality disorder in MZ than in DZ co-twins, although results remain significant when these subjects excluded. Verbal and visual episodic memory discriminates affected from unaffected co-twins, therefore familial factors necessary but not sufficient.
QTL analysis in family of affected individual		
(Tuulio-Henriksson et al., 2002)	Finnish isolate: 264 individuals from 131 families, 137 SZ or SZA, 34 other diagnosis, 93 no diagnosis	Backward digit span: H=0.42 (p=0.003), probability of at least one locus=73% Visual span: H=0.36 (p=0.06): probability of at least one locus (73% and 70%) Intrusions in verbal list recall: H=0.66 (p=0.001) Covaried for age, sex and verbal ability (H=0.62, p=0.0006). Best evidence for working memory, less strong for attention / long term memory functions.
QTL linkage and association studies using endophenotype approach in twins		
(Gasperoni et al., 2003)	Twin pairs discordant for SZ (30 DZ, 20 MZ), control twin pairs (27 DZ, 28 MZ)	Visual span linked to 1q41 locus, confirmed by allelic association. No linkage detected for other measures of frontal lobe function. Tested measures of frontal lobe functioning found to be sensitive to genetic load for SZ according to Cannon et al (2000)

Note

SZ = schizophrenia, SZA = schizoaffective disorder, BPD = bipolar disorder, SPD = schizotypal personality disorder, Aff = affective disorder

H = heritability, MZ = monozygotic, DZ = dizygotic, QTL = quantitative trait loci

4.1.5 Cognitive function in 22q11DS

Several investigations of cognitive function in children and children with 22q11DS have been conducted, and as yet no characteristic pattern of deficits has been detected. Early studies emphasised poorer performance IQ and visuospatial function with relatively spared verbal function, typical of a non-verbal learning disability (Swillen et al., 1997). This pattern was somewhat surprising given that speech and language impairment, occurring secondary to palate anomalies, velopharyngeal insufficiency and poor oromotor control, is a very common and often severe feature of the syndrome (Solot et al., 2000). Scherer et al (1999a) indicated that 22q11DS is associated with poorer speech and vocabulary learning than is the case for non-22q11DS children with equivalent palate anomalies, suggesting that development of communication abilities is vulnerable in 22q11DS for some reason other than structural anomaly. Additionally, autistic-spectrum disorders have been detected in children with the syndrome again suggesting that language and communication function is unlikely to be a spared domain (Niklasson, Rasmussen, Oskarsdottir, & Gillberg, 2001). Language dysfunction (independent of palate abnormality and general developmental delay) has been been reported in two studies using standardised language assessments and, in the latter case, an IQ-matched comparison group (Moss et al., 1999; Glaser et al., 2002).

There remains relatively little data on strengths and weaknesses in specific cognitive functions in 22q11DS. Bearden et al., (2001) reported impairment in visuospatial short-term memory relative to verbal task performance in school-aged children with VCFS. They suggest that this relative impairment contributes to mathematical disability in the syndrome. However, no comparison group was included in this study, and therefore no reason to believe that a similarly unbalanced profile, and concomitant relationship with poor mathematical skills, would not be seen in a group of non-22q11DS children with equivalently delayed development. More recent studies with IQ-matched comparison groups have failed to replicate the performance/verbal discrepancy as a syndrome-specific pattern, and have made little progress in detecting group differences in any cognitive test. In a study of 19 adults with VCFS compared to community-recruited learning disabled adults (Henry et al., 2002), significant group differences were detected in a test of visuospatial processing

involving identification of objects from unusual perspectives, and a complex planning task.

Although specific learning disabilities such as mathematical and visuo-motor impairment are much discussed in relation to the syndrome, there is little evidence that these domains are in fact vulnerable in 22q11DS to a greater degree than in other developmental disabilities. It may be that there is an as yet undetected "behavioural phenotype" for 22q11DS which does indeed define all individuals with the syndrome. However it is arguably more important to identify aspects of neurocognitive development which are rendered vulnerable in some but not all individuals with the deletion. No study has yet related variability in cognitive function to psychiatric outcome within a 22q11DS group.

4.1.6 Aims of this experiment

The primary aim of this part of the investigation was to establish any differences between 22q11DS subjects and IQ-matched controls on working memory tasks, similar to impairments seen in schizophrenia and in association with schizophrenia-risk. Experimental tasks were selected in order to broadly characterise any working memory deficit in terms of the Baddeley and Hitch tripartite model, i.e. to establish whether task performance varied as a consequence of the type of information (verbal, visuospatial) being processed or the demand placed on the central executive.

A second aim was to assess the impact of working memory variation within the 22q11DS group on language attainment. The relationship between neurocognitive impairment and language ability is pertinent in 22q11DS, because of the unusual pattern of speech and language development in these individuals, which cannot be fully accounted for by articulation impairments or learning disability (see Chapter 6 for further discussion of this pattern and its potential relationship to psychosis). Bagner, Melinder, and Barch (2003) showed that working memory was closely associated with language comprehension in schizophrenia. Therefore one hypothesis is that working memory dysfunction contributes to language impairments in 22q11DS by a similar mechanism as that seen in idiopathic schizophrenia.

A final aim was to establish whether there is any relationship between working memory abnormalities and either premorbid functional impairment or schizotypal personality features. A stable relationship between cognitive impairment and features akin to the negative syndrome is predicted within the schizotaxia model. However a relationship between working memory abnormalities and schizotypal phenomena is predicted within a neuropsychiatric model in which disruption to the neural substrate for cognitive control is directly associated with risk for positive symptoms such as hallucinations and delusional thinking. Evidence for both between-group differences in working memory performance, and within-group cognitive-behavioural relationships would establish working memory as a valid endophenotype for psychosis within 22q11DS, and thus a potential marker for genetic analysis.

4.2 Method

A short battery of cognitive tests was selected to assess a number of simple cognitive skills in a single session lasting no longer than an hour and a half (including tests of general ability). The majority of participants in this study had IQs in the mild or moderate learning disability range, and attention difficulties, thus it was essential to select tests that could be attempted beyond floor level by all participants.

4.2.1 Measures of working memory

Tests were selected in order to assess the integrity of components of the Baddeley and Hitch tripartite model of the working memory system. Digit span (forwards) from the WAIS-R was administered to measure phonological loop capacity. Subjects are presented aurally with strings of numbers of ever increasing length and must recall them in the identical sequence. The test is discontinued when a subject fails to recall either of two trials at any string-length. Two verbal tasks were administered that stress both the phonological loop and central executive components of the model - digit span (backwards) from WAIS-R, and sentence span as described by Siegel & Ryan (1989). The integrity of the visuospatial scratch pad was assessed by a simple paper and pen location-memory task.

In the sentence span task, participants must add a final word to complete simple sentences aurally presented by the investigator (for example, "cars have to stop at red.....", typical subject response - "lights"). Subjects must then recall their self-generated final words in trials of increasing numbers of sentences (from 2 to 5), in the order in which the items were produced. The test is discontinued when a subject fails to recall all self-generated items from a single trial (although not if all items are recalled but in the incorrect order). This test requires both sentence comprehension and verbal fluency during the completion phase, and working memory for final words during the recall phase. The sentence span task may place additional demands on inhibitory control processes, since final words must be selected in preference to non-final words. Such inhibitory control processes may require the recruitment on specific additional neural resources in the prefrontal cortex, a recruitment process that may be abnormal in schizophrenia. For the sentence span task, three recall scores were obtained; all items in a sequence recalled with order correct, all items in a sequence recalled but order incorrect, and total number of final-words recalled. In addition, an intrusions index was calculated. This is the number of non-final words produced at recall, divided by the total number of responses at recall (i.e. if in the course of the whole test, 20 items were recalled, 2 of which were non-final words, the intrusions index would be 0.1). This index was utilised (rather than raw number of intrusion errors) so as not to falsely increase or decrease the measurement of intrusion error as a function of stage of discontinuation of the test.

The visuospatial scratch pad was assessed by the Dot Test (Keefe, Lees-Roitman, & Dupre, 1997), a paper and pen task widely used in schizophrenia research. In this task, subjects are presented with a series of black dots, 0.5cm in diameter, each at a different location on sequentially presented white A4-sized sheets of paper. In eight no-delay trials, a blank A4 sheet of paper is placed beneath the stimulus, and subjects are asked to mark a dot on their piece of paper in the same pace as the dot in the stimulus booklet. In eight delay trials, each stimulus is presented for 5 seconds, followed by a 20 second delay during which subjects are asked to read as many words as possible from a sheet of simple distracter words. Then a blank sheet of paper is produced for the subject to mark the location of the dot on the previous stimulus page. For each of the 16 trials of the test, the distance (in mm) is measured between the edge of the stimulus dot and the centre of the subject-reproduced dot. The dot test score is calculated as the average distance from stimulus to response in

the delay condition minus the average distance from stimulus to response in the no-delay condition (to control for hand-eye co-ordination and motor ability). In addition, the number of responses produced at a distance greater than 5cm from the stimulus was counted (hereafter termed dot test failures), as this number perhaps reflects "absolute forgetting" of stimulus location whereas the average delay-distance reflects "approximate remembering" of location.

4.2.2 Measures of language and communication

Receptive vocabulary was assessed by the Quick Test (Ammons & Ammons, 1962) in which subjects select one of four complex pictures as being linked to single word stimuli. Up to 48 stimuli are presented (the pictures remain constant throughout), and the test is discontinued when subjects make the incorrect choice on four successive trials.

Expressive language was assessed using the Formulated Sentences subtest from the Children's Examination of Language Fundamentals (Semel et al., 1987). In this task, subjects are presented with a complex picture of a scene and a word (presented aurally by the investigator, one repetition allowed) on every trial, and asked to make up a sentence with the word in it, using the picture to help if they wish. In the last 5 trials, two words are presented to be included in the response sentence. The test is discontinued if the subject fails to generate a response (which need not be a complete sentence) containing the index word on four successive trials.

Participants' everyday language and communication ability was assessed by parental questionnaire, using the Children's Communication Checklist (Bishop, 1998). This consists of 50 items covering structural (articulation, phonology, syntax) and pragmatic language skills, yielding two index scores (speech, pragmatic). The pragmatic index score comprises several subscales (Inappropriate initiation, Coherence, Stereotyped conversation, Use of context, Rapport). Two final scales, Interests and Social, are included in the CCC to differentiate pragmatic language difficulty from other behavioural phenomena associated with more pervasive autistic spectrum disorders. The CCC was filled in by a parent or carer and returned to the investigator by mail.

4.3 Results

4.3.1 Working memory

Group mean performance scores for all working memory tasks are presented in Table 4.2. The distribution of all test scores in the 22q11DS group deviated from the normal distribution (Shapiro-Wilk statistic, $p < 0.05$). Non-parametric analysis indicated that, with the exception of forward digit span, there was a trend towards poorer performance in all tests in the 22q11DS subjects. However, only one task, the Dot Test of visuospatial working memory, revealed a significant group difference, with 22q11DS subjects making significantly more errors of greater than 5cm. Although this was the only significant between-groups effect, the trend towards significance for the verbal working memory tasks argued against a specific spatial working memory deficit in 22q11DS.

To test the hypothesis that a general working memory deficit exists in the 22q11DS group, a working memory index was compiled by averaging the whole-sample Z-scores for backwards digit span (phonological loop), dot test errors (visuospatial scratch pad) and sentence span total items correct (central executive). By computing a mean working memory score in this fashion, individual variance in task performance across the different tests will be cancelled out. If some subjects did very poorly on the Dot Test but well on the sentence span task, or vice versa, for reasons other than working memory capacity, then average Z-scores will converge on the whole-sample mean and no between groups difference on the working memory index would be predicted. The mean working memory index score for controls was 0.22 (s.d.=0.6) and -0.20 (s.d=0.8) for 22q11DS. Thus a wide range of working memory index scores was displayed by subjects in both groups. Index scores were normally distributed in the control group (Shapiro-Wilk $p=0.26$) but not in 22q11DS (Shapiro-Wilk $p = 0.044$) where the distribution was positively skewed (but not distorted by outliers). Non-parametric analysis indicated a significant group difference in working memory index scores (Mann-Whitney U, $Z=-2.2$, $p=0.028$). Since working memory index scores differed between groups but was highly correlated with IQ (whole sample Spearman's rho = 0.59, $p < 0.001$), univariate

analysis was carried out with IQ as covariate. This indicated no significant effect of group (F=2.1, p=0.15) once IQ had been accounted for.

Table 4.2 Working memory performance in 22q11DS and control groups

Working memory component	Test	Group: mean (s.d.)		*Mann-Whitney U*
		22q11DS	**Controls**	
Phonological loop	Digit span (forward)	6.4 (2.1)	6.3 (1.9)	*-0.2*
Phonological loop plus central executive	Digit span (backward)	3.5 (1.4)	3.9 (2.0)	*-1.0*
Visuospatial scratch-pad	Dot score (recall – copy)	2.4 (1.7)	1.8 (1.2)	*-1.4*
	Dot test failures	2.4 (2.0)	1.2 (1.3)	*-2.2 **
Central executive	Sentence span	3.75 (2.1)	4.5 (1.6)	*-1.0*
	Total number of final-words recalled	16.2 (8.6)	20.0 (6.7)	*-1.9*
	Sentence Span intrusions index	0.16 (0.15)	0.10 (0.10)	*-1.2*

*** p<0.05 ** p<0.01**

4.3.2 Language and communication

Tables 4.3 and 4.4 present the results of direct testing and questionnaire assessment of language and communication in 22q11DS and controls. Groups did not differ on receptive vocabulary attainment but did differ very significantly of tests of expressive ability and pragmatic language use. Since Formulated Sentences differed between groups but was highly correlated with IQ (whole sample Spearman's rho = 0.51, p<0.001), GLM univariate analysis was carried out with IQ as covariate. This indicated a significant effect of group (F=8.9, p=0.004) even once IQ had been accounted for. Additionally, since the 22q11DS group scored below controls on the CCC speech measure, GLM univariate analysis covarying for CCC speech was carried out to assess whether expressive language difficulties (Formulated Sentences score) can be accounted for by articulation difficulties and other speech production impairments in the 22q11DS group. This indicated that CCC speech score contributed a non-significant portion of expressive language variance (F=0.3, p=0.56), whilst the effect of group remained significant (F=6.3, p=0.017). This

result remained unchanged even when CCC speech and IQ are simultaneously entered as covariates.

Breakdown of the CCC pragmatics index into individual scales indicated significant group differences on three dimensions: coherence, stereotyped conversations and rapport. This suggests that adolescents with 22q11DS may have particular difficulty with complex social communication that relies upon mutual engagement in a topic of joint interest. They appear to have less difficulty in following the conventions of more formal interactions, indexed by scales of inappropriate initiation and use of context. This mirrors the experience of the investigator in communicating with 22q11DS study participants. Face-to-face or telephone conversations that involve standard script-like communication such as use of appropriate greetings and small-talk do not seem to be problematic, but more novel and personal conversations seemed less easy. Scores on the final two scales indicated that although the social interactions of the 22q11DS subjects were abnormal (limited peer interactions, preferring the company of adults), they do not in general show the type of idiosyncratic interests or repetitive behaviours seen in autism.

Table 4.3 Speech, language and communication in 22q11DS and control groups

Test	Group: mean (s.d.)		*Mann-Whitney U*
	22q11DS	**Controls**	
Quick test (raw score)	25.5 (6.4)	28.2 (4.6)	*-1.4*
Formulated sentences (raw score)	26.6 (14.4)	39.3 (9.3)	*-3.4* **
CCC speech score	28.6 (4.9)	33.6 (2.4)	*-3.5* **
CCC pragmatics index score	134.1 (11.6)	145.3 (13.4)	*-2.7* *

*** p<0.05 ** p<0.01**

Table 4.4 Pragmatic language skills in 22q11DS and control groups

CCC Pragmatics Index sub-scale	Group: mean (s.d.)		Mann-Whitney U
	22q11DS	Controls	
Inappropriate initiation	24.3 (3.7)	26.1 (3.4)	1.6
Coherence	29.4 (4.3)	33.2 (3.1)	3.0 **
Stereotyped conversation	23.7 (3.5)	26.7 (3.1)	2.7 **
Use of context	26.3 (3.8)	27.7 (4.6)	1.5
Rapport	28.8 (3.6)	32.0 (2.5)	3.3 **
Social	28.2 (3.9)	31 (3.6)	2.4*
Interests	29.6 (3.2)	30.8 (2.0)	1.3

*** p<0.05 ** p<0.01**

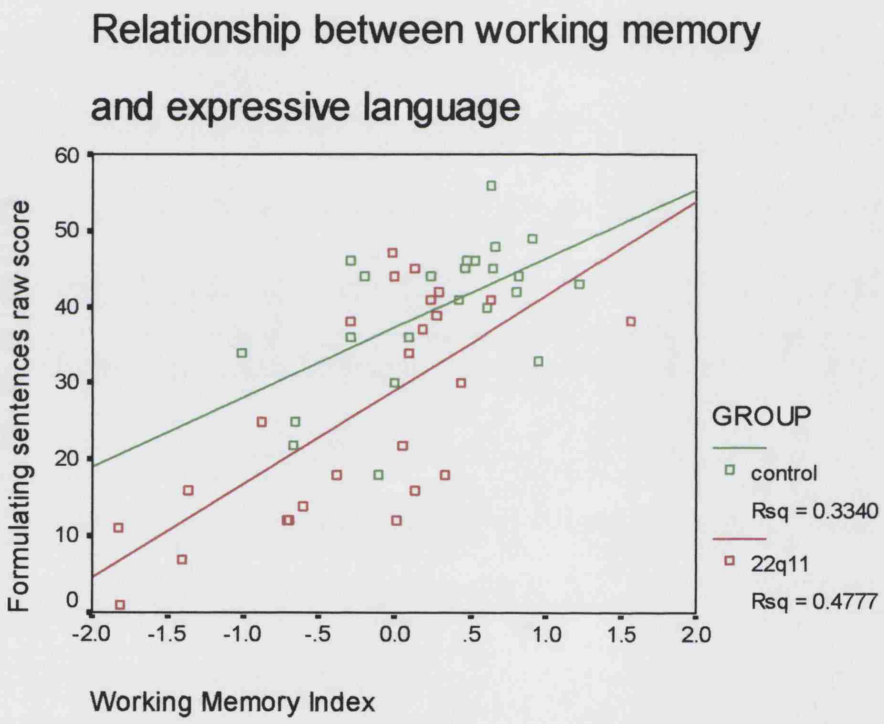
4.3.3 Relationship between working memory and language abnormalities

Spearman's non-parametric correlations were computed separately for the 22q11DS and control groups to assess the relationship between working memory abnormalities (using the summary working memory index) and language abnormalities (expressive ability and pragmatic use). This indicated a very strong relationship between working memory and expressive language ability (Formulating Sentences raw score) in both 22q11DS (R=0.64, p=0.001) and control groups (R=0.47, p=0.03). This relationship remained significant in partial correlations co-varying for IQ (22q11DS: R=0.58, p=0.006; controls: R=0.57, p=0.02). This indicates that a tight association exists between the neurocognitive functions assayed by working memory and expressive language tasks. One explanation for this is that working memory places specific constraints on language both during development, for example during vocabulary acquisition, and during on-line sentence construction and execution. An alternative explanation is that both working memory and language are "whole-brain" skills, requiring functional connectivity between multiple cortical and sub-cortical brain areas.

In contrast, working memory was not correlated in either group with pragmatic language ability as measured by the Children's Communication Checklist. This may

indicate that social communication is influenced by complex environmental factors such as communication within the family, speech and language therapy, and opportunities for supported social interactions with peers in educational and other settings, to a greater extent than pure linguistic ability. An alternative explanation is that a parent-report questionnaire measure is inaccurate and thus inappropriate for correlational analysis, since many factors will determine the nature of informants' response, including expectations of appropriate communication skill and opportunity to observe social communication in a variety of settings.

Figure 4.1



4.3.4 Relationship between cognitive impairment and psychiatric symptoms

A partial correlation, co-varying for IQ, was computed between scores on the schizotypy scale and working memory index score. This indicated a non-significant trend towards an association in 22q11DS subjects (partial R=-0.36, p=0.09). However, there was no similar relationship between expressive language function and schizotypy (R=-0.3, p=0.2) nor between pragmatic language function and

schizotypy (partial R=0.06, p=0.8). There was also no relationship between any cognitive variable and the total CAPA symptom score.

4.3.5 Relationship between cognitive impairment and premorbid adjustment

Partial correlations, co-varying for IQ, indicated a trend towards poorer premorbid adjustment scores in individuals with poorer working memory function in 22q11DS subjects (R=-0.37, p=0.08) but not in controls (R=-0.1). Interestingly, there was a significant relationship in both groups between working memory index scores and scores on the peer relationship PMA sub-scale (22q11DS R=-0.4, p=0.04; controls R=-0.53, p=0.01). This suggests that, during adolescence at least, the neurocognitive processes subserving working memory function mediate individual differences in some aspect of social interactions. A further set of partial correlations were computed between working memory and PMA scores, entering expressive language (Formulating Sentences) as a co-varying factor, to test the hypothesis that working memory impacts on social function via its association with communication ability. This rendered the correlation between working memory and PMA peer relationships non-significant in both groups, supporting this hypothesis.

4.4 Discussion

4.4.1 Summary

- The 22q11DS group did not differ from the control group on individual tests of working memory function (dot test error score was the exception to this rule) however there was a consistent trend towards lower scores in the 22q11DS group.
- A summary measure of working memory test scores, standardised to whole-sample performance, revealed a significant between-groups difference. This indicates that at least some of the 22q11DS subjects consistently performed more poorly than IQ-matched control subjects, in tasks involving working memory for both visual and verbal stimuli, with or without central executive demands. This suggests some degree of overlap between cognitive impairments in 22q11DS and individuals at-risk for schizophrenia in the general population.

- 22q11DS subjects also performed more poorly on a test of expressive language ability (Formulated Sentences), and received lower scores on a questionnaire measure of pragmatic language use (CCC). A strong relationship existed in both 22q11DS and control groups between expressive language and working memory, not accounted for by within-sample variance in IQ.
- Weak relationships were found between working memory performance and scores on both the schizotypy scale and PMA scale, suggesting that working memory does warrant further investigation as a cognitive domain potentially related to risk for psychosis in 22q11DS.

4.4.2 Limitations

- The measures used in this investigation did not allow for adequate assessment of the capacity of each component of the Baddeley working memory model. Further studies could employ N-back type tasks, presenting auditory, verbal and visual information in parallel experiments, with parametric variation of information load, delay-times and presence of distracters.
- The extent of expressive language dysfunction in the 22q11DS group warrants further investigation using more extensive testing protocols. The Formulated Sentences test may be criticised as a complex task reliant on many overlapping skills, including verbal and pictorial comprehension, memory, generative ability, grammatical knowledge and speech execution. As such, it provides only an estimate of general expressive ability in a formal setting, and cannot estimate either social language capability, or selective adequacy of component language skills.
- Relationships between cognitive and psychiatric measures were only tenable when IQ was entered into partial correlations as a co-varying factor. Thus is it difficult to establish the specificity of the relationship between performance on working memory tasks and psychiatric risk, independent of learning disability. Given the wide range of general ability levels in the 22q11DS group, an endophenotype that co-varies with IQ would not be useful for genetic analysis, since a sliding-scale of IQ-dependent scores would be required to define severity of disruption. Analysis of co-variance for working memory and IQ is unlikely to have the power to detect within-group dependence of the endophenotype on a

genetic factor because IQ will account for a very large proportion of variance in scores.

- It is arguable whether a behavioural measure will ever provide reliable, IQ-independent results in this population, given significant levels of both learning disability and psychiatric disturbance, both of which are likely to impact upon task performance in a non-specific fashion.
- Other cognitive domains not investigated here which could provide as strong or stronger evidence for schizophrenia-like disruption in 22q11DS include attention, executive function, episodic memory and social cognition.

4.4.3 Implications

Cognitive assessments revealed weak evidence for schizophrenia-like deficits in 22q11DS. On average, 22q11DS subjects were likely to perform less competently on working memory tasks than IQ and age-matched controls. However there was no evidence of a bimodal distribution of working memory scores within the 22q11DS group. Therefore quantitative and not qualitative differences in some factor underlying working memory task performance are likely to mediate within-group variation.

The lack of dissociation between performance on visuospatial and verbal tasks in the 22q11DS group (relative to control group performance) suggests an impaired cognitive process that is common to all tasks. This could be at the level of retention, organisation or execution of response, or in a low-level processing ability that is required for adequate function of both of the slave systems. The two tasks in which 22q11DS subjects scored most poorly, relative to controls, were Dot test and sentence span. A common feature of these two tasks is that they require retention of information over a delay period during which distracting (verbal) information is provided. This is also the case for digit span (late-presented items may obstruct retention and rehearsal of preceding items), but in this case the delay time is shorter and no information is presented which is not part of the response. Therefore a speculative interpretation of these results is that impairment resides within the "central executive" components of the Baddeley model.

The relationships between working memory and psychiatric status (schizotypy and premorbid adjustment) in the 22q11DS group indicate that quantitative differences in cognitive performance are accompanied by variation in psychological indicators of psychosis risk. Working memory disruption could be a valid endophenotype for psychiatric vulnerability in 22q11DS, in accordance with recent work showing that working memory is a powerful quantitative trait for liability to psychosis in the general population (Gasperoni et al., 2003). However, in a small population, using measures for which there is very considerable overlap in performance between at-risk and control populations, and for which there is also dependence on (or impact upon) general cognitive ability, resolving genotype-endophenotype relationships seems an unlikely proposition.

5 Auditory change-detection - a potential neurophysiological endophenotype for psychosis in 22q11DS

5.1 Introduction

5.1.1 Event-related EEG potentials – tools for investigating the dynamics of neural activity

Electroencephalography is a simple technique for eliciting dynamic information about central nervous system function. By averaging multi-lead scalp EEG traces in a time-locked manner, event-related potentials (ERPs) generated by the presentation of stimuli or by behavioural responses can be extracted. ERPs reflect synchronised changes in post-synaptic activity in populations of neurons at the cortical surface. Hence it is possible to obtain considerable information about the time-course of neural processing under different experimental conditions or in different populations. A certain amount of information can be obtained about the number and location of neural processing centres associated with the event-related potential (termed generators), with increasing accuracy dependent on the number of recording electrodes and the sophistication of analysis. However the spatial resolution of this technique is very much reduced in comparison with MRI or MEG (magnetoencephalography) and is limited by volume conduction through the brain, distortion by the skull and lack of signal originating from deep brain structures.

A great number of ERP studies have been conducted with respect to neural abnormalities in diverse diagnoses, the developmental emergence of ERP components, their cognitive and structural correlates in normal and atypical development, and their neurochemical regulation. A certain amount of information exists regarding the genetic architecture of individual differences in ERP components, although as yet there are no gene variants associated with ERP variability in the normal population or with ERPs that are potential risk markers for cognitive impairments or psychiatric illness. Therefore ERP methodology, and knowledge obtained via its use, presents a broad and powerful base from which to

integrate levels of analysis (molecular, neural and behavioural) in normal and atypical groups.

5.1.2 Neurophysiology of auditory processing and change detection

ERPs consist of a sequence of time-locked components in the EEG waveform, therefore are most easy to study and most informative when a cognitive process is itself clearly dependent on time at the millisecond scale. For this reason the auditory modality has been very intensively studied using ERPs, both to investigate auditory processing specifically and to investigate modality-general higher level cognitive processes such as attention and memory via auditory stimulation.

One of the core techniques in auditory ERP research is to present repetitive stimuli and to extract elements of processing by introducing rare stimuli (deviants) that differ in some respect to the frequently presented stimuli (the standard). This enables the comparison of ERP waveforms that are associated with repetitive stimulation by the standard with the deviant-elicited waveform. These very simple processes can be studied passively, with the subjects' conscious attention diverted to irrelevant distracter tasks or stimuli. The removal of task performance as a factor influencing neural function in a circuit of interest is an advantage when studying atypically developing populations with learning and behavioural difficulties. By varying stimulus-type (for example pure tones versus speech sounds) and contrast-type (for example duration, intensity or phonetic) it is possible to compare the topography and time-course of ERP components involved in processing different behaviourally-relevant stimuli. This enables comparisons to be made with regard to the structural and functional integrity of specific neural circuits within individuals and between groups. Some of the key properties of the auditory oddball ERP sequence are summarised in Table 5.1

Table 5.1a Characteristics of the auditory oddball ERP sequence: N1

	N1		
Definition	Automatic early response following presentation of repeated auditory stimulus		
Sub-components	N1a	N1b	N1c
Approximate latency post stimulus-onset (ms)	75	100	130
Maximal electrodes	Temporal	Fronto-central	Anterior / posterior temporal
L / R asymmetry	Bilateral, but bias may shift from right to left during adolescence	Bilateral	Bilateral?
Probable generator(s)	Auditory association cortex in superior temporal gyrus	Supra-temporal plane of auditory cortex	Not known
Cognitive correlates	Stimulus onset / offset	Pitch analysis?	Discrimination
Neurochemical regulators	Loudness-dependence modulated by serotonergic function.		
Developmental maturation	Progressive decreases to adult amplitude at 7-8 years. Earlier maturation on left than on right, for both tonal and speech stimuli.	Shifts from parietal to fronto-central maximum by late teenage years, reflecting maturation of tonotopic organisation of auditory cortex.	For tones only distinguishable from N1a at 11-12 years, but earlier for speech.
Association with developmental disorders	Possibly abnormal in sensorineural hearing loss, specific language impairment and autism.		
References	(Pang & Taylor, 2000)		

Table 5.1b Characteristics of the auditory oddball ERP sequence: MMN

	MISMATCH NEGATIVITY (MMN)	
Definition	Early attention-independent response to infrequently presented stimulus (deviant), which differs from the standard in a physical feature, or in the conjunction of features, or in an abstract feature of regularity.	
Sub-components	Temporal	Frontal
Time window in adults (ms)	Peaks between 130 and 200ms depending on deviant-type.	Peaks slightly later than temporal component.
Maximal electrodes (10-20)	Mastoids and central	Fronto-central
L / R asymmetry	Bilateral. Asymmetry may emerge for stimulus-type e.g. pitch change on right, speech on left (reports are inconsistent).	Bilateral, but sources may differ (see below).
Probable generator(s)	Superior temporal plane (Heschl's gyrus), slightly different locations dependent on stimulus-type and hemisphere.	Inferior frontal cortex (right), superior frontal gyrus, cingulate (left).
Stimulus-sensitivity	Amplitude increases, and latency decreases, with larger degree of difference between standard and deviant.	
Cognitive correlates	Comparison between standard and deviant.	Prelude to involuntary attention switching. May be involved in feedback "tuning" of auditory cortex.
Neurochemical regulators	Glutamatergic (ketamine) blockade at NMDA receptors reduces amplitude. Dopaminergic (haloperidol) blockade reduces latency and does not change amplitude.	
Developmental maturation	Amplitude declines with age.	Amplitude is mature by mid childhood. Peak latency declines with age.
Association with developmental disorders	Reduction association with dyslexia / genetic risk for dyslexia. No consensus regarding specific language impairment or autism.	
Association with neurological insult	Prefrontal cortex lesions reduce amplitude.	
References	(Naatanen & Winkler, 1999) (Jemel, Achenbach, Muller, Ropcke, & Oades, 2002) (Gomot, Giard, Roux, Barthelemy, & Bruneau, 2000)	

Table 5.1c Characteristics of the auditory oddball ERP sequence: P3

	P3	
Definition	Involuntary but attention-modulated cortical orienting response to infrequently presented target or novel stimulus. May reflect transition from covert to conscious processing.	
Sub-components	P3a	P3b
Time window in adults (ms)	After 280ms	Simultaneous to, or following, P3a
Maximal electrodes (10-20 system)	Frontal (Fz / Cz)	Posterior (Pz)
Probable generator(s)	Prefrontal cortex, and a distributed network involving temporal lobe structures (including parahippocampal gyrus) and anterior cingulate.	Temporo-parietal cortex, posterior hippocampus and posterior cingulate.
L / R asymmetry	Bilateral	Bilateral
Cognitive correlates	Evaluation of novelty and capture of attention for orienting toward novel stimulus (regardless of task relevance)	Orienting towards task-relevant stimulus
Neurochemical dependence	Haloperidol administration (dopamine blockade) reduces amplitude in normal volunteers.	
Developmental maturation	Amplitude increases and latency declines with age.	Latency declines with age.
Association with neurological insult	Dorsolateral prefrontal cortex lesions result in P3a-selective reduction	Temporo-parietal junction lesions result in non-selective P3 reduction
References	(Friedman, Cycowicz, & Gaeta, 2001), (Johnstone, Barry, Anderson, & Coyle, 1996), (Smith et al., 1990)	

5.1.3 Cognitive properties of auditory oddball ERPs

There is still a great deal of debate and uncertainty regarding the cognitive processes reflected by this type of oddball procedure and the waveforms that it elicits. However, by manipulating experimental parameters the simple oddball technique can be adapted to address very diverse theoretical questions. Some ways in which the properties of the oddball ERPs have been directly investigated and utilised as a marker for the outcomes of higher cognitive operations are summarised below.

5.1.3.1 Change detection and echoic memory

The deceptively simple cognitive operation of change detection is in fact dependent on sophisticated cognitive machinery and interactions between several sub-systems. Both perceptual discrimination and sensory memory functions are involved in making comparisons between each incoming stimulus and some existing neural trace activated by the preceding stimulus to generate an auditory stimulus representation for each sound object in the auditory scene (Naatanen et al., 1999). The N1-MMN-P3 sequence may reflect specific components of this complex process, although relationships between each ERP component, and between the ERP components and a corresponding set of cognitive process, are far from clear at present. The ERP sequence is sensitive to many aspects of the context in which a stimulus is presented. It is thus unlikely to reflect a purely sensory response to novelty and likely to be influenced by higher-order memory organisation for auditory objects and their expected co-occurrence (Winkler, Schroger, & Cowan, 2001).

The MMN component is thought by some investigators to reflect activity in echoic memory, allowing online processing and integration of information within a limited time window of several seconds (Cowan, Winkler, Teder, & Naatanen, 1993). This ability to hold information "in mind" for a short period may be particularly relevant for language comprehension, for example in understanding a sentence, since one must be able to maintain syntactic and semantic context in order to integrate many elements into a single meaning. One can speculate that this mechanism could have particular importance developmentally, for example with regard to word learning. An infant will require an extended window of time during which to pair an utterance

with the availability of a visual stimulus corresponding to the utterance which may not have been in the line of the infant's vision at the exact time of the utterance. For this reason and others the maturation of neural systems underlying ERP generation may occur in parallel to the emergence and maturation of information processing systems underlying language learning (although no inference of causality can be made).

A recent development has been the extension of MMN experiments to include the detection of deviants in complex patterns where there is no single standard stimulus but a repetitive block of stimuli that can be interrupted by a pattern-breaking but physically non-novel deviant. Similarly, the "standard" can be defined as an abstract rule, for example, the higher the frequency the louder the intensity (Paavilainen, Simola, Jaramillo, Naatanen, & Winkler, 2001). These experiments indicate the influence of top-down expectancy and complex analysis systems in the action of sensory memory and even low level perceptual processing, in-line with the view that a great deal of cognitive activity is generative rather than analytical (Friston & Price, 2001). This experimental paradigm is therefore of interest with regard to theoretical perspectives on psychosis, for example it may provide neuroscientific evidence for loosening of associations between immediate past and present events, or distinctions between self- and other-generated perceptions. At the most abstract level, MMN may provide a measure of the relative engagement of the individual with the internal and the external worlds.

5.1.3.2 Stimulus sensitivity and perceptual processing

By varying the type of deviant stimulus and degree of difference between the standard and deviant it is possible to address questions about selective perceptual acuity, and to examine parallel or convergent streams of information processing within the brain. Types of physical deviance within a sound, for example duration, frequency, intensity and location in space give rise to slightly different distributions of MMN-like responses across the scalp surface, suggesting partially distinct processing centres, as observed in MEG. However not all investigators have found topographic differences between responses to duration and pitch (Jemel et al., 2002), probably reflecting different stimulus and recording protocols. fMRI and ERP studies both point towards the existence of multiple neural centres contributing to the

standard trace, deviant-detection and comparative analysis, some of which may be feature-irrelevant or even modality-irrelevant and thus integrated with general attention and memory systems, and some of which may be feature-sensitive and thus integrated with other perceptual analysis systems (Stevens, Skudlarski, Gatenby, & Gore, 2000).

Although it is clear that the magnitude of the oddball responses increase with increasing degrees of physical separation between the standard and the deviant (Jaramillo, Paavilainen, & Naatanen, 2000), an issue that has yet to be fully resolved is how different deviant features within one stimulus are responded to in combination. Some experiments have found that different features are combined in an additive fashion as reflected in MMN amplitudes and latencies (Paavilainen, Valppu, & Naatanen, 2001) whilst others have argued that individual features and their conjunctions are processed in parallel (Takegata, Paavilainen, Naatanen, & Winkler, 1999). The P3 complex seems to have a much less predictable dependence on the magnitude of standard-deviant difference of feature conjunctions, and may reflect an "all-or-nothing" perceptual transition of information from pre-attentive to conscious processing (Dehaene, Sergent, & Changeux, 2003).

5.1.3.3 Temporal properties of stimulation and windows of integration

Varying time dimensions such as interstimulus interval or number of standards preceding each deviant allows investigation of the properties and capacity of memory systems such as the proposed temporal window of integration for sound objects and decay or reinforcement of learning of novel events. When an ISI* is very long, the sensory memory trace for the standard may decay such that a new sound has only a weak signal against which it can be compared, reducing the likelihood that it will be detected as a deviant. However when sounds are presented very close together they may be integrated into a single perceptual object (Sussman, Winkler, Ritter, Alho, & Naatanen, 1999). When the average number of standards between each deviant increases, another scale of time within memory systems is implicated. This increases the degree to which a deviant sound is unexpected and therefore processed as a change, resulting in the relative reductions in response to the standard (N1) and enhancement of MMN (Imada, Hari, Loveless, McEvoy, & Sams, 1993).

* interstimulus interval

5.1.3.4The influence of attention on auditory ERPs

By varying experimental conditions for example passive testing versus requirement of active detection of the deviant versus the performance of a distracting task unrelated to the oddball, one can investigate the role of attention on perceptual processing and distractibility. The availability of additional distracting auditory information, for example by playing a video with the sound on whilst participants undergo testing, tends to reduce the ERPs considerably (McArthur, Bishop, & Proudfoot, 2003), presumably by occupying the majority of "space" within available streams of processing. Application of attention towards the stimuli in a task-relevant manner does not reduce the MMN, but does increase the P3 components (in particular P3b) which can obscure or distort the MMN (Schroger & Wolff, 1998). By attending to the stimuli but not making the deviant behaviourally relevant the deviant-related ERPs may actually increase. Thus processes reflected in the oddball ERP sequence may be influenced by a functional balance between the processing of task-relevant information within working memory and the availability of additional neural resources for diverting attention towards task-irrelevant but potentially significant distractions (Berti & Schroger, 2003). This balancing act may fluctuate in time within an individual, via regulated neural or neurochemical modulators promoting either one or other process, and identification of such modulators could indicate potential sources of individual difference.

5.1.3.5Dynamic changes in auditory ERPs over time

Analysis of change in ERP components over the course of a testing session or over a longer period of time such as days, weeks and months is starting to illuminate the dynamics of neural processing associated with adaptive change and learning. In the course of a continuous testing session lasting between several minutes and several hours, temporal ERP components habituate and frontal components increase, suggesting a process of adaptive change that facilitates the processing of some information whilst inhibiting others (Baldeweg, Williams, & Gruzelier, 1999b). Naatanen and colleagues (1993) found that MMN paralleled the course of learning to discriminate change within a complex repeating sequence of tones. In this experiment, no adults initially generated deviant-related responses, but by the end of the session individuals who could behaviourally discriminate between standard and deviant patterns did generate an MMN, whereas those who could not do the

behavioural discrimination task never developed the change-related ERP. There are two opposing explanations for this result. One possibility is that the process of pattern-learning relies upon the degree of plasticity within the circuits involved in MMN generation, and therefore individual differences in this type of plasticity determine learning and cognitive flexibility at the behavioural level. A second possibility is that MMN is the automatic perceptual outcome that reflects learning processes driven by other neural circuitry. In either circumstance, MMN acts as a marker of individual differences in complex pattern learning. Oddball ERPs have also been used to track auditory learning over a longer period of time, for example during the learning of a non-native language (Cheour, Shestakova, Alku, Ceponiene, & Naatanen, 2002) and the development of sound discrimination after cochlear implants in deaf children (Ponton et al., 2000).

In summary, auditory change-related ERPs can be used as markers for the operation of many different but overlapping cognitive systems. There is a great deal of basic neuroscience yet to be completed with regard to these systems, and although this does not preclude the use of the marker as a tool for investigating normal and abnormal development it does indicate that caution should be taken with regard to experimental design and interpretation of findings.

5.1.4 Auditory oddball ERPs in schizophrenia

Central to the neurodevelopmental hypothesis for schizophrenia is the presumption that it is a neuronal illness, in which one should find structural and functional brain abnormalities both before and during symptomatic episodes. Similarly, an illness with a genetic origin must derive its pathophysiology from abnormal molecular mechanisms that act either during early development and / or across the life-span to regulate some aspect of normal biology. Whilst neuropsychological investigation can provide some information regarding disrupted neural substrate, more direct measures may be possible with structural and functional imaging techniques and neurophysiological assessment. In common with cognitive function, a very diverse number and type of functional brain anomaly has been uncovered in schizophrenia, in part reflecting the diversity of techniques and questions addressed and in part reflecting diversity of study populations.

Atypical ERPs reflect developmental or functional abnormality in specific neuroanatomical systems, although it is unlikely that there is a one-to-one mapping between structural abnormality and ERP change. These systems can be inferred from abnormal amplitudes, latencies and electrode distribution of responses. Components of the auditory oddball ERP rely upon intact structures in temporal and frontal lobe structures, and communication between these regions. Structural and functional abnormalities in these regions and in their connectivity have been frequently reported in schizophrenia (Dolan, Fletcher, McKenna, Friston, & Frith, 1999). Thus abnormal oddball ERPs would be expected to co-occur with these neuroanatomical abnormalities. Atypical ERPs may also reflect functionally abnormal or vulnerable systems of neurochemical regulation, for example in dopaminergic and glutamatergic modulation pathways, which can give rise to neurobiological disruption and symptoms under certain circumstances, and which may be ameliorated by pharmacotherapy (Javitt, 2000). There is a large literature pertaining to auditory processing ERPs in schizophrenia and this is selectively summarised in Table 5.2.

Additional inferences about underlying abnormalities in an index population can be made from altered sensitivity to specific stimuli. Relatively greater reductions in duration MMN than frequency or intensity MMN have been reported in schizophrenia, and there is some evidence that behavioural impairments in temporal-detection tasks co-occur with duration MMN deficits (Todd et al, in press). Further specification of deficits can be obtained via experimental manipulations such as varying stimulus onset asynchrony, presumed to test the duration of auditory sensory memory, or frequency of presentation of deviants which alters their salience and expectancy weightings. Baldeweg and colleagues (in preparation) have demonstrated that schizophrenia patients with memory impairments are insensitive to probability change, and fail to generate an MMN at frontal electrodes under any circumstances. On the other hand patients with intact memory skills are sensitive to probability change to the same extent as controls, although responses are smaller in amplitude. This suggests that a subgroup of patients, who show a chronic course of illness, may be specifically unable to evaluate stimuli in a manner necessary for behaviourally-relevant learning. This could in turn explain why language and memory abnormalities are seen in association with the illness in some cases but not others.

Disruptions in change-detection, echoic memory and the attribution of salience to external stimuli may contribute directly to the anomalous experiences which underlie the emergence of symptoms such as hallucinations and delusions, although no ERP experiments have yet been conducted to test this theory. Evidence for a specific relationship between neural abnormality (e.g. MMN reductions) and positive symptoms (e.g. hallucinations) has not been forthcoming. Alternative evidence is provided by a study that manipulated ERPs in normal individuals by pharmacological challenge and then asked subjects to report associated psychological phenomena. (Umbricht et al., 2000). This indicated that healthy volunteers under the influence of ketamine (an NMDA antagonist) experienced psychosis-like symptoms but only if the drug induced reductions in MMN amplitude. This only occurred in individuals with high schizotypy questionnaire scores, who were presumably more vulnerable to experiencing psychosis-like phenomena. These fascinating results point towards a tight interdependency between individual capacity for neurophysiological regulation, personality-type and vulnerability to symptoms. Thus ERPs may be an important tool for constructing empirically-driven integrated models of psychosis-proneness.

By observing the brain responding to simple changes in sound patterns, we may start to understand what the brain is doing when it is "making order from chaos" (Ivry & Knight, 2002) and thus what underlies the lack of order and increased chaos that may contribute to schizophrenia. By investigating individual differences in this process, and in its dependence on neurochemical and even psychological modulators (e.g. social stress) as detrimental and compensatory factors, our understanding of the schizophrenia process may be greatly enhanced.

Table 5.2 Characteristics of auditory oddball ERPs in schizophrenia

	Auditory ERP Component		
	N1 in schizophrenia	MMN in schizophrenia	P3 in schizophrenia
Amplitude	Inconsistent results. Less facilitation at short SOA could indicate reduced accuracy of temporal integration. (Todd, Michie, Budd, Rock, & Jablensky, 2000)	Reduced. Meta-analysis (Krljes and Umbricht 2003) indicates mean effect size of ~1.36.	Usually reduced, but dependent on active / passive paradigm, performance, duration of illness, medication.
Electrode distribution: asymmetry	Left-right balance associated with active / withdrawn syndromes (Gruzelier, 1999)	Reduced current density on left but not right side (Youn, Park, Kim, Kim, & Kwon, 2003)	Stimulus-dependent asymmetry retained (Kayser et al., 2001)
Electrode distribution: topography		Reduction in frontal component > temporal (Baldeweg, Klugman, Gruzelier, & Hirsch, 2002)	
Stimulus-sensitivity	Intensity-dependence lost in depression / OCD (Hegerl & Juckel, 1994)	Deficit more pronounced and consistent for duration than pitch deviants (Michie et al., 2000a)	
Medication-sensitivity	Reduction in medicated patients only?	Generally no effect detected.	Clozapine improves (Umbricht et al., 1998)
Association with symptoms	Associated with all symptom cluster scores (Todd et al 2000)	Left-sided reduction association with positive symptoms (Youn et al 2003), although most reports find no association.	Associated with negative symptoms.
Association with cognitive dysfunction		Associated with memory impairment in chronic patients.	Associated with general cognitive dysfunction.

5.1.5 Auditory oddball ERPs as potential endophenotypes in genetic research

Any stable quantitative parameter that reflects an aspect of neurodevelopmental integrity and is associated with risk for psychiatric disorder might be useful as a marker for genetic variants predisposing to illness. Neurophysiological measurements are particularly good candidates for endophenotypes, because they may directly reflect neuronal function and be sensitive to genetically-regulated aspects of cellular function. This statement needs to be qualified with considerable pure neuroscience research, since little is known at present about the relationships between, for example, changes in synaptic plasticity or neuronal conduction efficiency, and altered scalp-recorded ERPs. Progress in identifying and characterising auditory processing abnormalities that may be endophenotypes for schizophrenia are summarised in Table 5.3.

layer 2/3 dendrite density?

There is moderate evidence for heritability of components of the auditory oddball ERP sequence although studies to date suffer from lack of specificity with respect to ERP components. The evidence that both MMN and P3 could be valid indicators of illness-risk is strong but requires extension and clarification. The two components may be independent markers of different functional clusters of genetic risk factors. Only one study (Michie et al 2002) has so far measured both components in one high-risk population, finding that MMN but not P3 abnormalities were present in unaffected relatives whereas both were present in patients. Studies of schizophrenia-spectrum disordered populations also indicate that P3 abnormalities may be present only in individuals who express some degree of psychosis-like phenotype in addition to being carriers of genetic risk (Salisbury et al 1996, Kimble et al 2000).

Specificity of ERP abnormalities as markers of risk for specific psychiatric diagnoses is debatable. On the one hand, MMN reductions are reported for schizophrenia but not for bipolar disorder, suggesting a high degree of specificity (Umbricht et al., 2003). On the other hand, MMN deficits are also seen in alcoholism and in developmental disorders such as dyslexia and language impairment. There are several possible explanations for these findings. Each disorder may in fact be associated with a distinct pattern of ERP abnormality (in terms of topography stimulus-specificity, modulation by attention) which has yet to be completely specified. Alternatively, disorders showing common patterns of ERP abnormality

may share neurodevelopmental risk factors and even genetic risk factors. A common neurodevelopmental endophenotype may lead to any one of multiple disorders (or none) depending on interactions with other endophenotypic features and environmental influence.

Table 5.3 Evidence in support of auditory ERPs as endophenotypes for schizophrenia

Reference	Study population	Summary of findings
CRITERION 1 – ASSOCIATION WITH ILLNESS RISK		
Illness study (first episode)		
(Brown, Gonsalvez, Harris, Williams, & Gordon, 2002)	40 SZ (first-episode), 40 SZ (chronic), two normal control groups (age and sex matched)	Differences in ERPs to both targets and non-targets in both SZ groups. Used discriminant analysis to classify patient groups by ERP characteristics
Test-retest stability in face of clinical change		
(Shinozaki et al., 2002)	13 SZ patients, acute exacerbation vs. post-acute symptom improvement, normal controls.	MMN amplitude at Fz reduced at both time points. MMN amplitude at mastoid electrodes reduced during acute illness only, and correlated with BPRS symptom score.
(Mathalon, Ford, & Pfefferbaum, 2000)	36 male SZ assessed at multiple time points (between 2 and 7 occasions), 34 controls.	Auditory (but not visual) P300 is stable across time, (66-72% trait variance) Patients appear to fluctuate about their own average P300, amp correlating with BPRS
Specificity for schizophrenia		
(Catts et al., 1995)	11 unmedicated SZ, ? medicated SZ, 11 bipolar (medicated), multiple age- and sex- matched control groups.	Reduction in duration MMN in both medicated / unmedicated SZ. No MMN reduction in BPD. Duration increment more discriminating between groups than duration decrement.
Multiple studies cited in (Frangou et al., 1997)		N200 and P300 abnormalities also reported for alcoholism, affective disorders and dementia.
Spectrum study – schizotypal personality disorder		
(Salisbury, Voglmaier, Seidman, & McCarley, 1996)	11 male DSM-III-R schizotypal personality disorder vs. 11 controls.	Asymmetrical P3 in SPD (reduced over left temporal lobe).
Spectrum study – psychometrically defined schizotypy		
(Nuchpongsai, Arakaki, Langman, & Ogura, 1999)	40 high- (psychosis-prone) vs. 40 low-scoring college students on Chapman scales	Reduced N2 and P3 amplitudes, and longer latencies in psychosis-prone group.

Reference	Study population	Summary of findings
Family study – adult relatives		
(Michie, Innes-Brown, Todd, & Jablensky, 2002)	22 SZ patients, 17 first-degree relatives, 21 controls.	Reduced MMN amplitude to duration deviant in patients and relatives. P3a larger in relatives than patients.
(Jessen et al., 2001)	11 SZ patients, 15 first-degree relatives, 16 controls.	Reduced frontocentral MMN amplitude to pitch deviant in relatives but not patients. Latency longer in patients (but not sig diff.)
(Frangou et al., 1997)	33 SZ, 57 non-SZ (including 10 unaffected "obligate carriers" of high genetic risk) from 16 families, 32 controls	Latency and amplitude reductions of N200 and P300 waves in relatives and patients. Do not specifically report data for obligate carriers
(Blackwood, St Clair, Muir, & Duffy, 1991)	Non-SZ members of large multiplex pedigrees (151), unrelated SZ (96), unrelated healthy controls (212).	P300 amplitude and latency abnormal in ~ 30% high-risk family members.
(Kimble et al., 2000)	15 first-degree relatives vs. 15 controls, also assessed for schizotypy (equivalent number of high-schizotypy subjects in both groups).	P300 amplitude influenced both by family status and by schizotypy
Family study – high risk children		
(Schreiber, Stolz-Born, Kornhuber, & Born, 1992)	21 high-risk children, 21 controls.	Reduction in frontal difference wave (Nd) and P3 to attended targets, but not MMN to unattended targets.
Prospective study – high risk sample		
(Squires-Wheeler, Friedman, Skodol, & Erlenmeyer-Kimling, 1993)	Adolescent offspring of SZ, affective disorder, no diagnosis. Followed up at age 25.	P3 decrement (auditory and visual modalities) correlates with Global Personality Functioning at follow-up, but non-specific for parental group or individual diagnosis.
CRITERION 2 – HERITABILITY		
Twin study (general population)		
(O'Connor, Morzorati, Christian, & Li, 1994)	59 MZ pairs, 39 same-sex DZ pairs.	Evidence for independent heritability of global target / non-target ERP characteristics, of latency / amplitude of N1 and P3 components, and of distributed ERP components with fronto-central topography.
(Katsanis, Iacono, McGue, & Carlson, 1997)	30 MZ pairs, 34 DZ pairs	Genetic influence on P3 latency and amplitude, and amplitude of N1, P2 and N2.

Reference	Study population	Summary of findings
Twin study (affected-unaffected)		
(Weisbrod, Hill, Niethammer, & Sauer, 1999)	MZ pairs: 5 concordant for SZ, 8 discordant, 9 control.	P300 reduced in affected and unaffected SZ co-twins Pitch contrast adjusted to individual discrimination-ability.
Sibling study (affected-unaffected)		
(Karoumi et al., 2000)	21 SZ patients, 21 non-SZ siblings, 21 controls.	Prolonged latency of P300 in both patients and unaffected siblings. Reduced amplitudes in patients only.
Linkage within multiply-affected family		
(Blackwood et al., 2001)	Family members within a high-risk pedigree (for SZ and affective disorders).	Family members carrying a translocation at 1q42 display P300 abnormalities regardless of penetrance / type of psychiatric diagnosis.
QTL analysis in sample high-risk for alcoholism		
(Porjesz et al., 2002)	Large sample of families with high-density of alcohol dependency	QTLs identified for P300, N100 and N400 in a visual paradigm. Suggestive linkage for each component on several chromosomes.

Note
SZ = schizophrenia, BPD = bipolar disorder, SPD = schizotypal personality disorder
MZ = monozygotic, DZ = dizygotic, QTL = quantitative trait loci
BPRS = Brief Psychiatric Rating Scale

5.1.6 Auditory oddball ERPs in 22q11 Deletion Syndrome

Cheour and colleagues has conducted a small series of ERP experiments in children and young infants with 22q11DS. These studies showed that children with 22q11DS whose phenotype includes cleft palate show reduced MMN amplitudes, especially at long interstimulus intervals (Cheour et al., 1997) but that these deficits were also found in non-syndromal cleft palate children albeit to a lesser degree (Cheour et al., 1998). This was interpreted as an indication of reduced duration of auditory sensory memory traces in children who have speech production difficulties, and that children with additional learning impairment (as is the case in 22q11DS) are at particularly high risk for perceptual deficits and concomitant language and literacy impairment. However an alternative explanation is that the neurophysiological deficit in 22q11DS is unrelated to speech production difficulties but is related to disruption of relevant neural circuitry by a primary genetic mechanism.

5.1.7 Aims of this experiment

The detection, by Cheour et al, of a deficit in auditory change-detection ERPs in 22q11DS indicates that this neurophysiological system may provide us with an endophenotype for psychosis in this population. However, many questions remain unresolved. Those that will be addressed in this investigation are listed here:-

1. Does central auditory processing disruption in 22q11DS persist through childhood into adolescence and adulthood or does it resolve by late childhood?
2. Is a neural processing abnormality still observable when comparing individuals with 22q11DS to individuals of equivalent general cognitive ability (i.e. mild / moderate learning disability)?
3. Are long inter-stimulus intervals necessary to observe the reported deficit?
4. Does the impairment extend across different stimulus types (tone contrasts in duration and frequency, speech sounds) and across different degrees of physical separation between the standard and the deviant?
5. Does the impairment show any specificity for ERP components (N1, MMN and P3a) or for topographical distribution (frontal and temporal responses, hemispheric lateralisation)?
6. Does impairment show any dynamic sensitivity i.e. are the same impairments present in the first and second half of a testing session?

In the following sections, the experimental design and method will be presented. This is followed by pilot data from a group of normal adults, test-retest data to establish reliability, and developmental data to demonstrate the normal trajectory of maturational change of ERP components. Finally, the results of the 22q11DS-control comparison study are presented.

The purpose of the pilot-retest study was to determine the properties and stability of the oddball ERP sequence in a normal adult population using these stimuli and presentation parameters. Establishing experimental and individual subject reliability is vitally important when establishing that any measurement reflects a constant at-risk state. Whilst many illness-relevant aspects of brain function must be highly labile (in order to account for episodes of relapse and remission), measurements that do not display at least moderate individual reliability cannot be used in genetic

studies which involve small numbers of subjects and large numbers of variables and are thus highly susceptible to false positive associations. On the other hand, group-level experimental stability may be sufficient to address more descriptive questions relating to potential neural mechanisms underlying behavioural disorder.

Data from the test-retest study was combined with data from normal comparison populations for the 22q11DS and SLI studies in order to construct developmental trajectories for each ERP component. This assists in the interpretation of group-differences when comparing populations. If a group or an individual displays responses that conform to a pattern seen in younger children then it can be speculated that a developmental process is delayed rather than deviant. Only longitudinal data can actually confirm such a speculation. A second reason to construct developmental trajectories is simply to increase our understanding of the biology of the cognitively relevant neural processes under investigation. A response that is stable from a very early age is likely to play a different role from a response that either does not appear until adolescence or that is present during early development and then disappears.

5.2 Method

5.2.1 Experimental aims

A paradigm was designed to test the integrity of several functional properties of the central auditory processing system. Previous studies in schizophrenia, 22q11DS and other developmental disorders are difficult to interpret because they usually present only one or two stimulus contrasts during the course of an experiment. This can lead to an artificial sense that the complex neural circuitry underlying change detection is either "intact" or "impaired". It is possible that certain aspects of the system can be independently compromised whilst others may compensate for vulnerability, as suggested by Gruzelier (2003) to explain the atypical patterns of neuropsychological and neurophysiological function seen in some individuals with schizophrenia-spectrum disorders.

Duration sensitivity, as a time-dependent phenomenon, may involve activation of a distributed network in the temporal and prefrontal cortices in conjunction with subcortical structures such as the cerebellum and basal ganglia, whilst spectral information such as frequency deviation may be more dependent on the peripheral auditory apparatus and fine-grained tonotopic organisation of the auditory cortex. In addition to activating different perceptual centres, duration sensitivity may require a higher degree of truly comparative, echoic memory processing than pitch sensitivity which could be achieved via sensory mechanisms alone. A consequence of the separation of these systems may be a differential impact of deficits in detecting one or other feature on language development and psychological function. There is some evidence that duration MMN may be especially impaired in patients with schizophrenia (Michie et al 2000), although deficits in other deviant types have also been found.

Secondly, the mismatch negativity and P3a components of the oddball ERP are usually found to reflect the magnitude of the difference between the standard and deviant stimulus. This magnitude sensitivity may be necessary for accurate memory-based classification, reflected in the MMN, and for appropriate regulation of the orienting response, reflected in the P3a.

5.2.2 Experimental design

Three blocks of stimuli were presented to every subject – one pure tones, two speech (described in Chapter 6). Each block consisted of a single standard stimulus, and three deviants. Two deviants in each block differed from the standard in respect of a single parameter (the "single" deviants), whilst the third differed from the standard in respect of two or more parameters (the "multiple" deviants). Block order was randomised. The total probability of deviant stimulus was 9%, giving a frequency of 3% for each individual deviant within a block. Each deviant appears 60 times within a block of 2000 stimuli. Onset-onset time was 500ms. Stimulus order was randomised, with no fewer than 2 standard stimuli separating each deviant. Stimuli were presented binaurally via speakers (except in the first pilot experiment when headphones were used).

Sine-wave tone stimuli comprised standard (frequency 600kHz, duration 50ms) and deviants in duration (600kHz, 100ms), frequency (650kHz, 50ms) and duration + frequency (650kHz, 100ms). Rise-time was constant at 10ms. Tones were generated in Cool Edit '96.

5.2.3 Testing procedure and data processing

Stimuli were presented via Presentation Version 0.40 (Neurobehavioral Systems, Inc.), with event-codes dispatched to the recording system simultaneous to stimulus presentation. Intensity was calibrated using a Type 4165 microphone (fast peak measurement, A-weighting, K factor 0.3) and stimuli were presented at a constant intensity of 70db. Subjects watched a self-selected silent film throughout the testing session. Recordings took place in an electrically shielded, sound-proofed booth, with recording equipment in an adjoining room (except during the first pilot experiment when recording took place in the same room as the experimental equipment and investigator, without shielding). Recordings were acquired via SynAmps amplifier with system band pass 0.05-100Hz and a digital sampling rate of 500Hz. Continuous EEG was acquired via NeuroScan (version 4.2) and saved for later analysis.

In the initial pilot experiment Ag / AgCl electrodes were individually applied to the scalp with adhesive paste according to the International 10-20 system. EEG was recorded continuously from 11 scalp sites (A1, A2, F3, F4, Fz, C3, C4, Cz, P3, P4,

Pz). Vertical eye movements were monitored via electro-oculogram (EOG) recorded from electrodes placed above the right eye and below the right outer canthus. Recordings were referenced to the nose tip and a ground electrode was placed on the forehead. Electrode sites were prepared with alcohol and NuPrep to reduce scalp impedance. In all subsequent experiments, recordings were taken from 28 ring electrodes embedded in an Easy-fit cap and filled with Lectron II conductivity gel, and both vertical and horizontal EOG was recorded. The additional electrode locations were Fp1, Fp2, F7, F8, Ft7, Ft8, Fc3, Fc4, T7, T8, Tp7, Tp8, Cp3, Cp4, P7, P8 and Oz. Ground and reference electrodes were unchanged.

The data processing procedure was uniform for all recordings in this investigation. At least 20 blink artefacts of greater than 10% deviation were selected as templates from the continuous EEG file and additional blinks were automatically identified, individually inspected and selected for entry into the Neuroscan blink reduction regression algorithm. Data was then filtered off-line with a band pass of 0.5-25Hz and epochs obtained locked to stimulus-presentation time. Epochs were 600ms long with a pre-stimulus baseline of 100ms. Following baseline correction to average pre-stimulus amplitude, epochs with amplitudes exceeding +/-100 μV were rejected. Average waveforms were computed for each stimulus-type, and MMN waveforms obtained by subtracting the within-block standard from each deviant.

5.2.4 Data analysis

Following visual examination of grand average waveforms for each group, individual measurements of peak amplitudes, peak latencies and rectified areas were obtained automatically within fixed peak-detection windows and inspected to ensure true capture of individual peaks. Fairly wide peak-detection windows were chosen so as to accommodate inter-subject and developmental variation in latency of ERP components; N1 subcomponents were detected between 80 and 200 ms, MMN between 100 and 250ms, and P3a between 200 and 400ms post stimulus-onset. A drawback of measuring within such wide time-windows is that area measurements (estimating the magnitude of the standard-deviant processing difference indexed by the MMN) are imprecise and include both positive and negative deflections in the waveform that are outside of the actual time-window of the individual subject ERP.

5.2.4.1 Data presentation

The following conventions are applied to tables presenting ERP data. Labelling of tables and figures has been kept to a minimum for clarity. Electrode locations refer to the International 10-20 System. Units are microvolts (for peak amplitudes) and milliseconds (for peak latencies). Area measurements refer to the rectified area (summed integrals above and below the 0μV baseline irrespective of polarity) under the MMN subtraction waveform in the specified time-window. Group values are always presented in the form mean (standard deviation). GLM repeated measures analysis is the primary statistical procedure for assessing within-subject and between-groups effects. Greenhouse-geisser corrected degrees of freedom and Bonferroni-corrected post-hoc p-values are quoted where appropriate. All waveforms presented in figures are the grand average ERPs for the whole experimental group in question. Specific ERP components (N1, MMN and P3a) have been labelled in initial test-retest figures only.

5.2.5 Additional experimental procedures for case-control studies

Pure tone auditory detection thresholds for both ears were obtained at 250Hz, 1000Hz and 4000Hz, using a calibrated audiometer.

Following the EEG recording, behavioural discrimination for all contrasts presented was tested in a minimal pairs procedure. Six blocks of stimuli were presented: (tones) pitch, (tones) duration, (tones) pitch + duration, (speech) voicing, (speech) place of articulation, (speech) multiple. There were 24 pairs of stimuli per block, half of the trials being "same" pairs and the other half being "different". Each stimulus pairing in the speech blocks (for example ta – da) was presented 6 times in total (3 in one order and 3 in the reverse), whilst each tone contrast (for example short – long) was presented 12 times in total. Stimulus-onset asynchrony was 500ms for each pair (equivalent to the EEG stimulation protocol). Subjects responded verbally ("same" or "different") after each pair of stimuli, and the experimenter recorded the response by pressing left or right mouse button, sending responses to the presenting computer and simultaneously triggering the next stimulus presentation (with a 250ms silent pause between triggering a trial and stimulus presentation). Response files were imported into Excel and number of correct / incorrect responses obtained for each contrast type.

A small number of subjects found this task very difficult to understand and attend to, getting confused between matching pairs of sounds and matching sounds from sequential trials. If subjects performed at or below chance in the first condition of either the speech or tone task, the test was discontinued. In order to give participants a chance to practice the task, the first speech block (multiple phonetic feature contrasts) and first tone block (double-deviant contrasts) were excluded from analysis. "Hit rate" scores were calculated, defined as the % correct detection of "different" trials, minus % false alarms ("same" trials classified as "different").

5.2.6 Pilot / reliability study participants

Eleven adults (6 males, two left-handed) took part in the pilot experiment. Mean age was 29.7 (range 20 – 52). Data from one subject was excluded due to poor impedance and low number of acceptable trials for each stimulus type. 9 study participants were available for a second recording session, for confirmation of group-level results and individual test-retest stability of responses. Time between sessions ranged from 112 days (3.7 months) to 170 days (5.6 months), with mean time between sessions of 137 days (4.5 months). Paired sample t-tests were used to identify systematic inter-session differences. Paired sample (Pearson's) correlations were obtained to assess the intra-subject stability of each parameter.

5.2.7 Developmental study participants

Data was collected from 32 individuals between the ages of 8 and 30, comprising the retested adult sample and comparison samples for the SLI and 22q11DS studies. Details of recruitment and demographics of the child / adolescent comparison samples are provided in Chapter 2. All recordings used the same stimuli and measurement apparatus, data processing protocol and analysis procedures, except that double-deviants were excluded for the two youngest age-groups in order to reduce the length of the testing protocol. The ERP measures found to be stable within-subject, plus other key parameters of interest, were assessed for age-related change. Both correlational and age-group analyses were carried out. For the latter analyses, subjects were divided into five groups; Group 1: ages 8 – 10 (n=6), Group 2: ages 11-13 (n=6), Group 3: ages 14-15 (n=7), Group 4: ages 16-21 (n=6) and Group 5: ages 22-27 (n=7).

5.3 *Preliminary Results*

5.3.1 Pilot and reliability study

5.3.1.1 N1 responses to standard tone

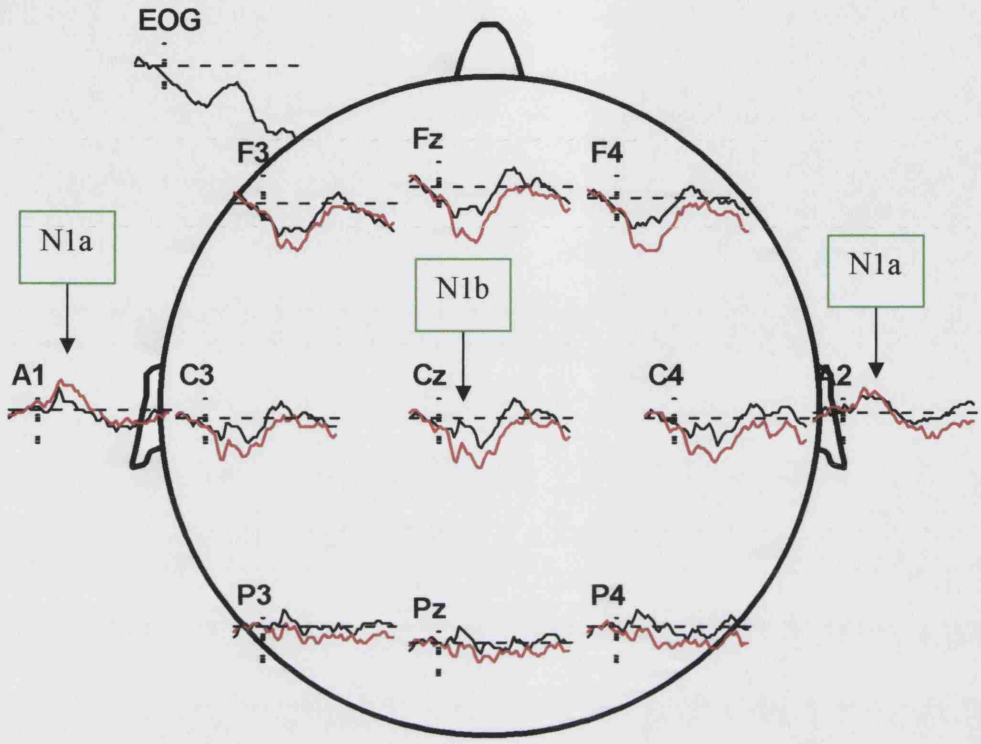
N1-type responses to the standard tone stimulus were small at both the temporal and frontocentral electrodes, with no significant hemispheric asymmetry for either amplitude or latency of the early temporal N1a component. N1c was not evident as a distinguishable peak for the majority of adult participants during either testing session or at either hemisphere. Potential reasons for the small size of these responses are the age of the subjects (N1 amplitudes decline with age reaching a steady state by mid-adolescence), the length of the recording session (responses may have habituated) and the short, regular inter-stimulus interval (N1 increases when stimulus presentation is unpredictable). Despite the small amplitudes obtained, responses were highly stable across the two testing sessions, with no significant increases or decreases at the group level, and significant within-subject correlations for N1a peak amplitudes and latencies on the right side and for N1b peak amplitude.

Table 5.4 N1 elicited by standard tone (pilot and retest)

Component	Electrode		Pilot	Retest	*Paired-sample t-test*	*Pearson's R*
N1a	A1	amplitude	-1.2 (0.6)	-1.3 (0.7)	*0.25*	*0.17*
		latency	87 (6)	87 (7)	*0.043*	*0.23*
	A2	amplitude	-1.0 (0.6)	-1.4 (1.2)	*1.4*	*0.76 **
		latency	88 (7)	90 (7)	*-1.4*	*0.78 **
N1b	Fz	amplitude	-1.3 (0.8)	-0.9 (0.8)	*-2.1*	*0.76 **
		latency	102 (11)	103 (16)	*-0.4*	*0.27*

*** p<0.05 ** p<0.01**

Figure 5.1 ERP waveforms elicited by standard tone (pilot and retest)



KEY TEST RETEST

5.3.1.2MMN responses to deviant tones

All three pure tone deviants generated a clear MMN, maximal at frontocentral electrodes and inverting bilaterally at mastoid and temporal electrodes (Figures 5.2 and 5.3). The most stable response was generated by the duration deviant, which elicited a pronounced frontocentral MMN which correlated well within subjects at test-retest and did not differ in magnitude at the group-level between sessions. The frequency deviant elicited a smaller MMN which was non-significantly reduced at session 2 relative to session 1 and showed no within-subject subject correlations. Responses to duration and frequency deviants recorded at the right mastoid showed a very high degree of within-subject stability in terms of peak latency (but not amplitude or area), which indicated that the speed of processing for single-feature contrasts may be a source of stable individual difference. MMN elicited by the double deviant was unstable at both the individual and group levels, perhaps because of the increase in the magnitude of the P3a response to the double deviant in session 2, which may have distorted the MMN deflection.

There was no evidence for hemispheric asymmetry of either the amplitudes or latencies of the responses measured at temporal electrodes. GLM repeated measures ANOVA analyses combining data from all three deviant-types obtained during test and retest sessions were performed to assess asymmetry of mastoid-recorded MMN. This revealed no main effects of laterality and no interactions between hemisphere and deviant-type.

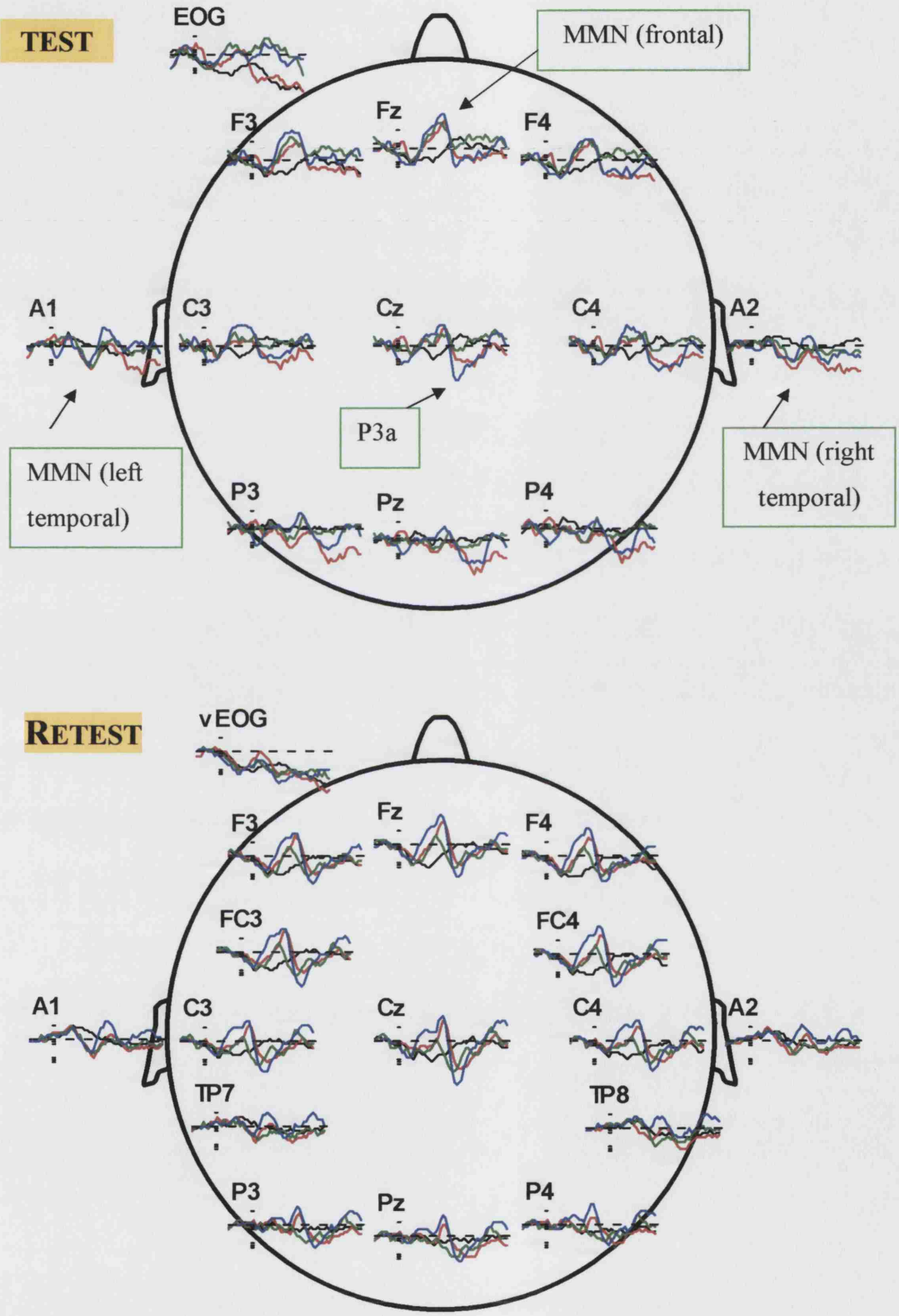
To examine the relationship between MMN measured at frontocentral and temporal (mastoid) electrodes, repeated measures ANOVA was conducted to investigate a potential latency shift between frontocentral and mastoid peaks, entering data from all three deviant- types (combining test and retest data). This indicated a significant latency shift between responses at Fz and A2 ($F[1,24] = 5.8$, $p=0.024$), with mismatch responses at the temporal electrode peaking earlier. This provides some indirect evidence that these two waveforms are dissociable and may be at least to some extent dependent on different neural generators. These results are consistent with those reported by other investigators, in particular Rinne et al (2000) who conducted a combined MEG / ERP study to model the neural sources of the MMN and reported latency differences between temporal and frontal components.

Table 5.5 MMN elicited by deviant tones (pilot and retest)

Deviant	Electrode		Pilot	Retest	*Paired-sample t-test*	*Pearson's R*
Duration	Fz	amplitude	-5.4 (3)	-5.6 (2.7)	*0.61*	*0.65 (p=0.058)*
		latency	185 (31)	186 (28)	*-0.02*	*0.46*
	A2	amplitude	3.4 (1.3)	3.0 (1.3)	*0.63*	*-0.11*
		latency	177 (25)	176 (24)	*0.53*	*0.95* **
Frequency	Fz	amplitude	-4.9 (2.0)	-3.8 (1.7)	*-1.2*	*0.03*
		latency	181 (24)	168 (18)	*1.6*	*0.3*
	A2	amplitude	2.9 (1.2)	2.4 (1.7)	*0.7*	*0.17*
		latency	172 (27)	164 (35)	*1.2*	*0.85* **
Duration + frequency	Fz	amplitude	-6.8 (3.6)	-5.8 (2.0)	*1.3*	*-0.61*
		latency	189 (30)	178 (23)	*-0.6*	*0.57*
	A2	amplitude	2.8 (2.0)	2.7 (1.9)	*0.03*	*-0.30*
		latency	161 (30)	154 (25)	*0.65*	*0.30*

*** p<0.05 ** p<0.01**

Figure 5.2 ERP waveforms elicited by tone deviants (pilot and retest)

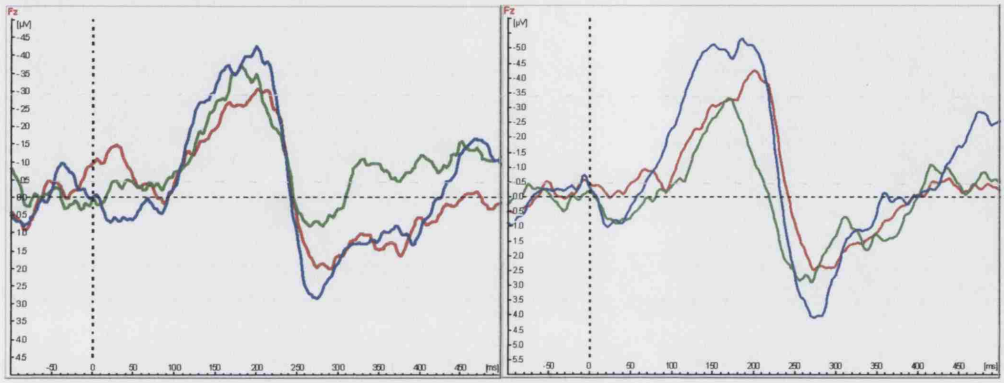


KEY STANDARD DURATION DEVIANT FREQUENCY DEVIANT
DURATION + FREQUENCY DEVIANT

Figure 5.3 Tone MMN (pilot and retest)

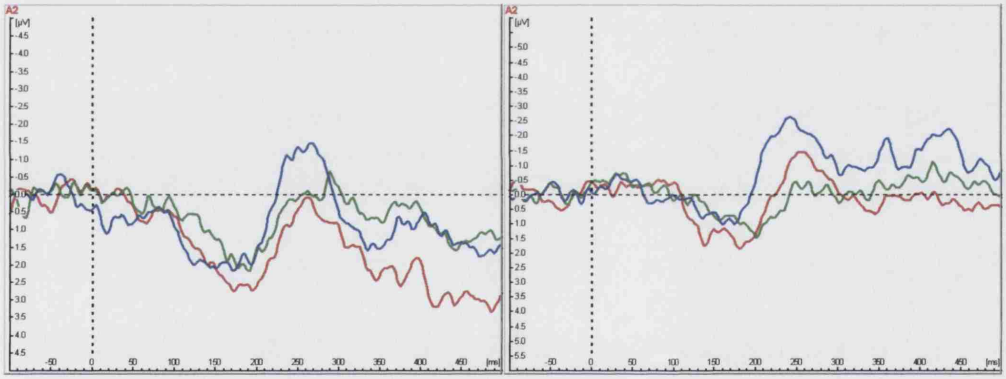
FRONTAL ELECTRODE FZ

Test **Retest**



TEMPORAL ELECTRODE A2

Test **Retest**



KEY DURATION DEVIANT FREQUENCY DEVIANT

DURATION + FREQUENCY DEVIANT

5.3.1.3P3a responses to deviant tones

Individual subtraction waveforms (deviant minus standard) were assessed with respect to the P3a component via automatic detection and measurement of peak amplitude and latency in the time-window 200-400 ms at the maximal electrode according to the group mean average waveforms (Cz). Repeated measures ANOVA revealed a main effect of session for P3a response latency ($F[1,24]=8.8$, $p=0.007$) but not for P3a peak amplitude ($F[1,24]=2.5$, $p=0.125$). This suggests that the P3a component is very sensitive to attentional state, since the response was enhanced by changing the recording conditions. Changes between sessions that could possibly account for this enhancement include moving from a light to a dark testing room, moving from a testing room in which the experimenter was present to a testing booth in which the subject was alone, and moving from headphone presentation to free-field presentation of stimuli. Despite the group-level increase in P3a amplitude between sessions, there were weak within-subject correlations for the amplitude of the response to each stimulus.

Table 5.6 P3a elicited by deviant tones (pilot and retest)

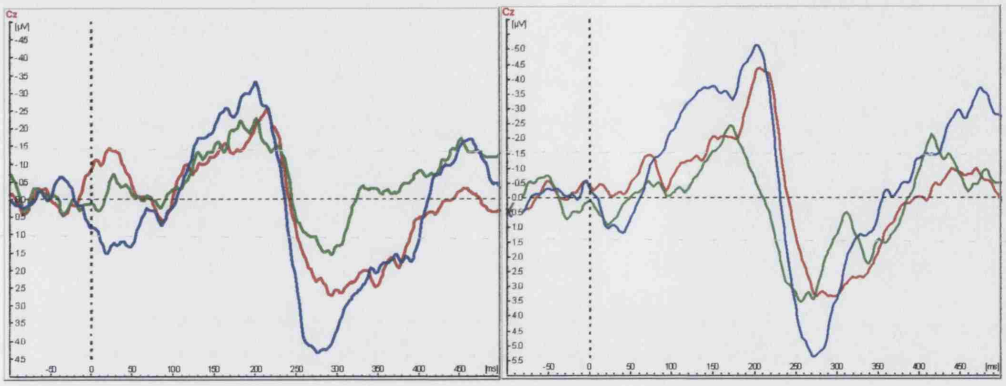
Deviant	Electrode = Cz	Pilot	Retest	*Paired-sample t-test*	*Pearson's R*
Duration	amplitude	4.5 (2.6)	4.9 (3.8)	*-0.27*	*0.168*
	latency	347 (68)	299 (38)	*1.8*	*-0.9*
Frequency	amplitude	2.3 (2.0)	4.1 (1.9)	*-2.6 **	*0.48*
	latency	353 (73)	315 (53)	*2.2*	*0.69**
Duration + frequency	amplitude	5.7 (3.2)	6.9 (4.9)	*-0.8*	*0.47*
	latency	283 (22)	269 (11)	*1.4*	*-.37*

*** $p<0.05$ ** $p<0.01$**

Figure 5.4 Tone P3a (pilot and retest)

ELECTRODE CZ

Test **Retest**



KEY DURATION DEVIANT FREQUENCY DEVIANT

DURATION + FREQUENCY DEVIANT

5.3.1.4 Contrast-sensitivity of tone MMN and P3a

To assess whether the ERP response to the double deviant (duration plus frequency) differed from the single deviants (duration or frequency alone), repeated measures ANOVA was carried out, entering data from test and retest sessions as within-subjects factors (Table 5.7).

The double deviant elicited a frontocentral MMN that was larger (in terms of area under the MMN curve within the MMN time-window) than that elicited by either of the single deviants. However it was not strictly speaking additive, as it was smaller than the sum of the magnitude of each individual deviant. Since there was no effect of deviant-magnitude on the peak amplitude of responses and there was some observational evidence of a double-peaked response within the frontocentral MMN for the double deviant, this response may reflect a summation of responses in independent generators with different response latencies.

There was no evidence for parallel magnitude sensitivity at the temporal electrodes, providing additional evidence for a different set of neural contributions to the responses at temporal and frontal sites. The P3a responses also display sensitivity to contrast magnitude, being larger and significantly earlier for the double deviant,

although post-hoc analysis indicates that this difference is only significant with respect to the frequency to double deviant contrast.

Table 5.7 Effect of contrast magnitude on tone ERPs (pilot and retest)

Component	Electrode		GLM repeated measures: effect of contrast magnitude			
			ANOVA F	*p*	*Post hoc comparison (Bonferroni corrected p- value)*	
MMN	Fz	amplitude	2.4	0.108		
		latency	0.6	0.568		
		area	5.0	0.015	Df > freq	0.016
					Df > dur	0.1
					Dur = freq	1.0
MMN	A2	amplitude	0.6	0.55		
		latency	1.2	0.31		
		area	1.3	0.28		
P3a	Cz	amplitude	3.0	0.07	Df > freq	0.068
					Df = dur	0.66
					Dur = freq	0.75
		latency	5.2	0.013	Df < freq	0.017
					Df < dur	0.064
					Dur = freq	1.0

5.3.2 Developmental trajectories

5.3.2.1 Development of tone N1

Both components of the T-complex (N1a and N1c) continue to mature through adolescence into adulthood, with steadily declining amplitudes and latencies. This is in line with previous reports (Pang et al., 2000) and may represent the increased efficiency (tuning) of sensory processing within the auditory cortex, increased inhibition of the auditory cortex by the prefrontal cortex, or reduction in processing that is necessary for development of neural representations but thereafter redundant. It was also noticeable that the P2 component, following the N1 complex, declined in magnitude with age.

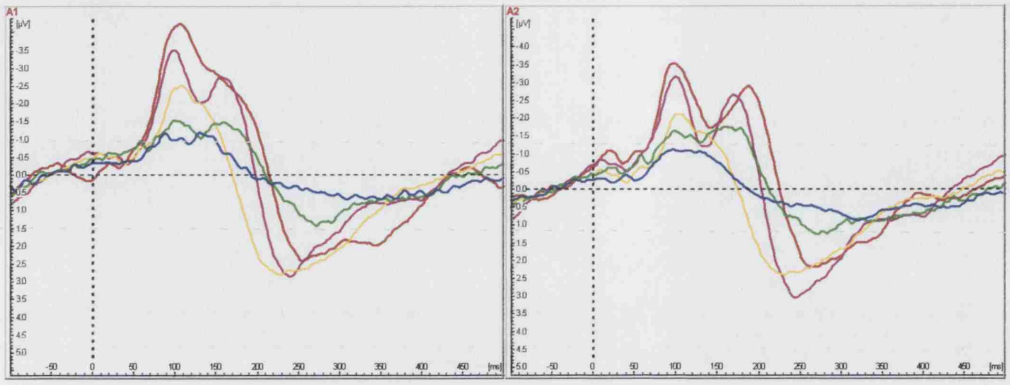
Table 5.8 Development of tone N1

Component	Electrode		Age group					Kendall's tau-b (age-group)	*Pearson's Correlation (age)*
			1	2	3	4	5		
N1a	A2	amplitude	-4.5 (0.9)	-3.2 (1.3)	-2.4 (1.2)	-1.8 (0.6)	-0.9 (0.6)	0.69**	*0.8* **
		latency	101 (7)	95 (10)	98 (11)	92 (16)	89 (8)	-2.7 *	*-0.4* *
N1b	Fz	amplitude	-2.2 (1.4)	-2.1 (1.2)	-1.3 (0.8)	-2.2 (0.8)	-1.0 (0.6)	0.27	*0.4* *
N1c	A2	amplitude	-4.1 (2.1)	-3.1 (2.5)	-1.2 (0.8)	-2.3 (1.8)	-0.8 (0.8)	0.40 **	*0.48* **
		latency	172 (21)	170 (13)	151 (3)	166 (16)	158 (14)	-0.21	*-0.24*

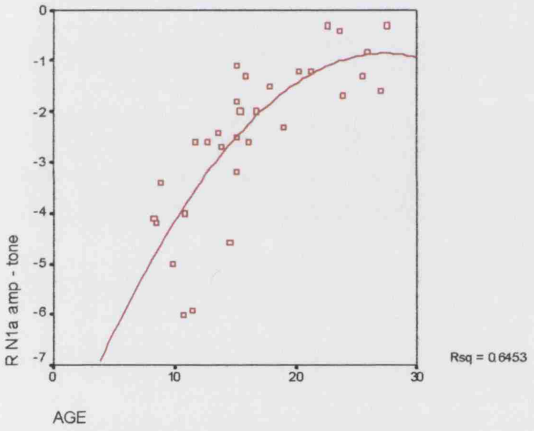
*** p<0.05 ** p<0.01**

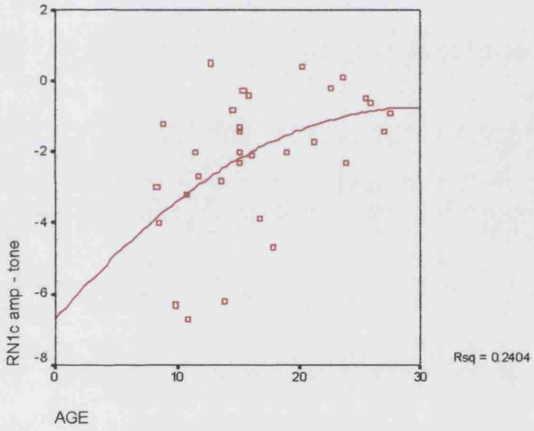
Figure 5.5 Development of tone N1

ELECTRODE A1 ELECTRODE A2



KEY GROUP: **1** ages 8 – 10 **2** ages 11-13 **3** ages 14-15 **4** ages 16-21 **5** ages 22-27





5.3.2.2 Development of tone MMN

Duration MMN recorded from frontal electrodes shows no consistent age-related change from late childhood, indicating relatively early maturation. Frequency MMN displays some age-related change (significant increases over late childhood for area measurements, trend for peak amplitudes) with shorter peak latencies and larger areas in adulthood than childhood. This indicates that sensitivity to small frequency changes may be a relatively late maturing phenomenon, occurring after the language system is well established. Additionally, the morphology of frequency MMN shows a late maturational pattern, with the youngest age group displaying a positive deviant-related ERP in the MMN time-window. For both duration and frequency MMN there was some evidence for increase in the magnitude of responses through adolescence followed by decline in early adulthood. This could indicate that certain neurobiological parameters reflected in these ERP components may undergo specific processes of change during the pubertal and post-pubertal period. Further investigation in a larger sample is needed to replicate and extend this observation.

MMN recorded at mastoid electrodes shows steady age-related decline through adolescence, for both duration and frequency deviants. This contrast with the steady-state of frontal MMN is in line with previous reports (Gomot et al., 2000) and may indicate once again at least partial independence of generators for the two topographically defined ERP components. The contrast in stimulus-specificity of maturational course between the two electrodes (frontal frequency MMN maturing later than duration, shared maturational course at temporal electrodes) suggests the existence of a common neural generator for the temporal responses, diverging to different processing systems for each contrast-type as reflected in frontal MMN.

Table 5.9 Development of tone MMN

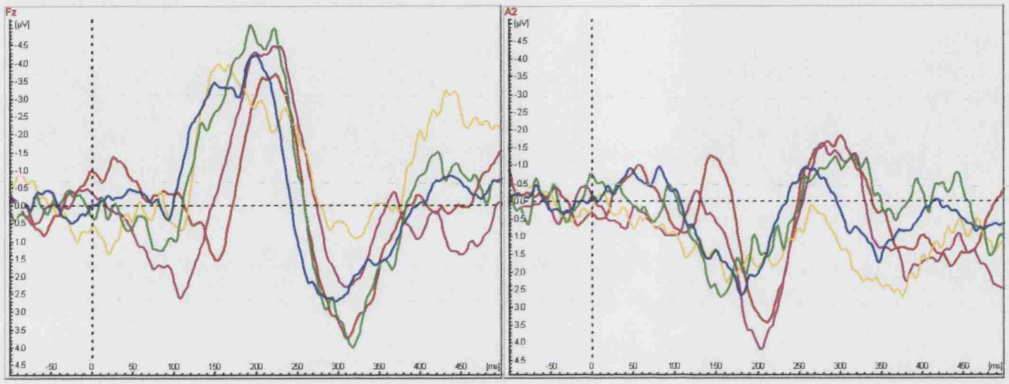
Deviant	Electrode		Age group					*Kendall's tau-b (age-group)*	*Pearson's Correlation (age)*
			1	2	3	4	5		
Duration	Fz	amplitude	-3.7 (2.4)	-5.9 (2.4)	-6.7 (3.5)	-7.4 (3.1)	-4.5 (2.3)	*-0.08*	*-0.07*
		latency	212 (11)	198 (28)	173 (41)	204 (23)	192 (24)	*-0.16*	*-0.13*
		area	325 (185)	607 (159)	569 (257)	542 (227)	453 (226)	*0.08*	*0.06*
	A2	amplitude	5.7 (1.6)	6.4 (3.5)	5.4 (2.7)	3.6 (1.4)	3.0 (1.1)	*-0.38 ***	*-0.49***
		latency	213 (14)	201 (36)	158 (27)	176 (28)	199 (24)	*-0.19*	*-0.13*
		area	464 (201)	561 (321)	554 (264)	261 (129)	242 (90)	*-0.36 ***	*-0.44 **
Frequency	Fz	amplitude	-2.8 (1.4)	-3.9 (2.0)	-4.5 (2.8)	-4.7 (2.5)	-3.7 (1.8)	*-0.1*	*-0.18*
		latency	166 (74)	179 (48)	191 (45)	182 (27)	178 (23)	*-0.02*	*0.03*
		area	214 (71)	309 (202)	409 (212)	400 (280)	384 (133)	*0.26*	*0.41 **
	A2	amplitude	3.1 (3.4)	6.8 (3.8)	4.8 (2.3)	3.8 (1.8)	1.8 (1.4)	*-0.25*	*-0.33*
		latency	229 (29)	229 (37)	173 (35)	183 (21)	175 (31)	*-0.45 ***	*-0.53 ***
		area	448 (305)	448 (279)	447 (296)	332 (87)	117 (69)	*-0.33 **	*-0.47 ***

*** p<0.05 ** p<0.01**

Figure 5.6 Development of tone MMN

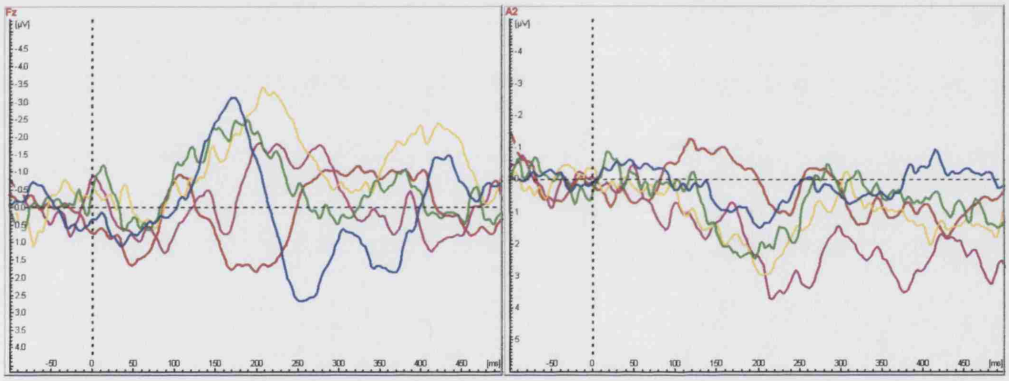
DEVIANT = DURATION

ELECTRODE FZ ELECTRODE A2

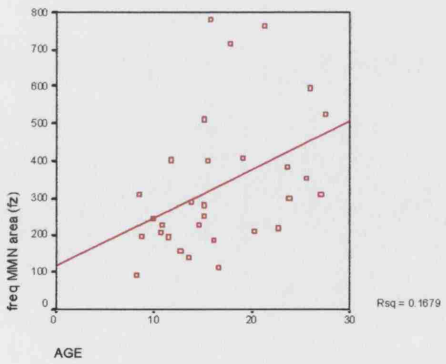


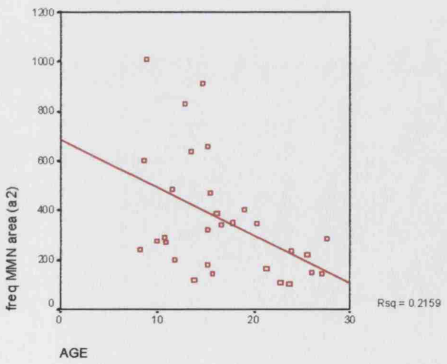
DEVIANT = FREQUENCY

ELECTRODE FZ ELECTRODE A2



KEY GROUP: 1 ages 8 – 10 2 ages 11-13 3 ages 14-15 4 ages 16-21 5 ages 22-27





5.3.2.3Development of tone P3a

There were no systematic age-related changes in the magnitude or latency of the P3a elicited by either duration or frequency deviants. This indicates that this basic attention-alerting mechanism may mature very early (large novelty responses analogous to the P3a are present in very young infants) in line with its potential importance as a developmental tool for orienting the young child towards sources of potential new information about the world. Unlike other ERP components that may be specifically necessary during the early developmental era (perhaps the temporal MMN falls into this category), the P3a is maintained into adulthood, indicating its continuing importance for information processing. Although individual measurements did not indicate any age-related change, group mean waveforms suggested that P3a was elicited more consistently in the older subjects. The system reflected by P3a activation may therefore become increasingly well integrated with sensory and working memory systems by adulthood.

Table 5.10 Development of tone P3a

Deviant	Electrode = Cz	Age group					*Kendall's tau-b (age-group)*	*Pearson's Correlation (age)*
		1	2	3	4	5		
Duration	amplitude	3.7 (2.6)	6.0 (4.4)	5.2 (3.2)	6.1 (7.7)	5.3 (2.6)	*0.08*	*0.15*
	latency	321 (46)	331 (38)	310 (41)	313 (41)	344 (69)	*0.02*	*0.13*
Frequency	amplitude	3.8 (2.2)	4.9 (1.5)	2.3 (2.3)	4.2 (4.5)	4.0 (2.8)	*-0.02*	*-0.04*
	latency	292 (71)	361 (46)	290 (26)	307 (66)	294 (57)	*-0.02*	*-0.16*

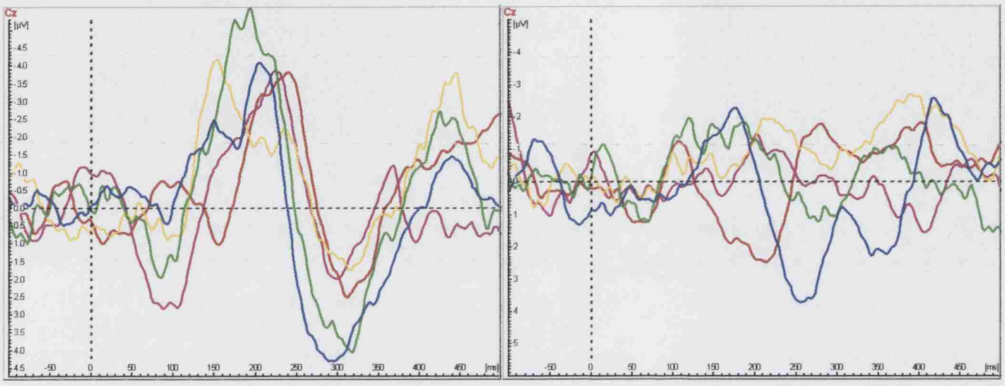
*** p<0.05 ** p<0.01**

Figure 5.7 Development of tone P3a

ELECTRODE CZ

DEVIANT = DURATION

DEVIANT = FREQUENCY



KEY GROUP: **1** ages 8 – 10 **2** ages 11-13 **3** ages 14-15 **4** ages 16-21 **5** ages 22-27

5.3.3 Summary of preliminary results

5.3.3.1 Stimulus-sensitivity and magnitude sensitivity of MMN and P3a

- A similar sequence of change-detection ERPs were elicited for all tone contrasts, comprising bilateral positive deflections at temporal electrodes and a frontocentral negative peak in the time-window 100-250ms (MMN), followed by a central positive peak in the time-window 250-400ms (P3a).
- In adults, the magnitude and latency of responses to duration and frequency deviants were equivalent, but the double deviant (duration + frequency) elicited a larger, double-peaked MMN response at frontal electrodes, and a larger P3a response of shorter latency.

5.3.3.2 Test-retest reliability

- Pure tone oddball stimulation for the elicitation of auditory ERPs is a moderately reliable procedure. Reliable N1 responses and moderately reliable MMN responses were generated at both frontocentral and mastoid electrodes, both in terms of peak amplitudes and latencies and in terms of individual intra-class correlations.
- The amplitudes of responses to duration deviants were much more stable at the individual level than were responses to either a frequency or double deviant. This may have been because the 100% duration increment was a very large acoustic deviation (although it is not perceptually very large), or because the processing of timing cues is a very robust neurophysiological phenomenon because of the necessity of binding events into a coherent time-frame.

5.3.3.3 Developmental trajectories

- The magnitude of responses to the standard tone (N1 complex) declined steadily through late childhood and adolescence. This may reflect either permanent tuning of neuronal populations, or increasing suppression of the response to a highly repetitive stimulus during the course of the testing block.

- A dissociation was observed between the maturational course of MMN amplitude recorded at frontal and temporal electrodes, the former being stable or increasing with age, and the latter declining.
- There was no consistent relationship between age and the latency of either N1 or MMN peaks.
- Frequency MMN appeared to mature more slowly than duration MMN. This may indicate the separation of neural generators responding to pitch and temporal change. On the other hand, the pitch deviant used in this experiment may represent a perceptually smaller standard-deviant contrast, thus dependent on mature auditory processing for accurate detection.
- The P3a component did not show any consistent relationship with age, either in amplitude or magnitude.

5.3.3.4Topographical distribution of MMN

- Several factors differentiate MMN responses recorded at the frontocentral and mastoid electrodes. They show a consistent latency difference (A2 peaking before Fz), a different profile of contrast sensitivity (only frontocentral responses are sensitive to contrast magnitude), and they mature at different rates (frontocentral MMN showing stability by late childhood for frequency and duration, mastoid responses becoming smaller with age through adolescence). Taken together, the evidence for different neural components contributing to MMN as detected at frontal and temporal electrodes is strong.
- An important question remains, however, as to the functional significance of each component for cognitive processing. One way in which this can be partially addressed is by seeking dissociations between components in atypically developing groups, and relating intact and impaired components to performance on cognitive tasks that may be predicted to rely upon one or other functional component of the change detection system.

5.3.4 Limitations of preliminary results

- The adult sample investigated here was not ideal for the establishment of test-retest reliability for the between-groups investigations because of the different ages of the samples, and the fairly low degree of variability between response magnitudes of individual participants.

- An additional limitation of the reliability analysis is the considerable variation in testing conditions between sessions 1 and 2. The high degree of similarity between responses at the group level indicates that the ERP components under investigation are not unduly influenced by environmental manipulations.
- The developmental sample did not contain children younger than age 8 and it would be interesting to extend the sample further into early childhood.
- The developmental sample included adolescents with mild / moderate learning disability, recruited as control subjects for the 22q11DS study. This may have distorted the developmental trajectories and may explain some of the contrasts between our developmental data and that previously published, for example with regard to P3 latency. However, since no relationships were detected between IQ and N1/MMN measures in any group contributing to this investigation, this is unlikely to be a major confounding factor.
- Many issues relevant to the auditory change detection system are not examined within this paradigm, which may be relevant both to schizophrenia and to developmental disorders. These include effects of attention to the stimuli, interstimulus interval, probability of deviant, stimulus location and intensity, feature conjunctions as opposed to standard-deviant magnitude, and responses to deviation in abstract patterns.

5.4 Case-control results

5.4.1 Audiometry

79% of 22q11DS participants and only 17% of controls showed evidence of mild hearing impairment (average pure tone detection threshold between 20 and 40dB). No participant displayed an average threshold above 40dB, the standard cut-off for moderate hearing loss. Average pure tone thresholds were higher in 22q11DS than in controls (Mann-Whitney U, $Z = -3.0$, $p<0.05$). Mean thresholds were higher than controls at all frequencies, with no evidence for an interaction between group and frequency. It should be noted that the testing conditions under which the audiometric measurements were taken were not ideal; a limited range of frequencies were presented, number of presentations of each stimulus were not standardised, and no repeat measurements were taken. The group difference in thresholds was more apparent for the left ear than the right, and since the left ear was always tested first this suggests that that the 22q11DS group had more difficulty than controls in comprehending the task instructions and focusing sufficiently to detect the very quiet sounds. Therefore this data can only be considered as an estimate of actual hearing thresholds.

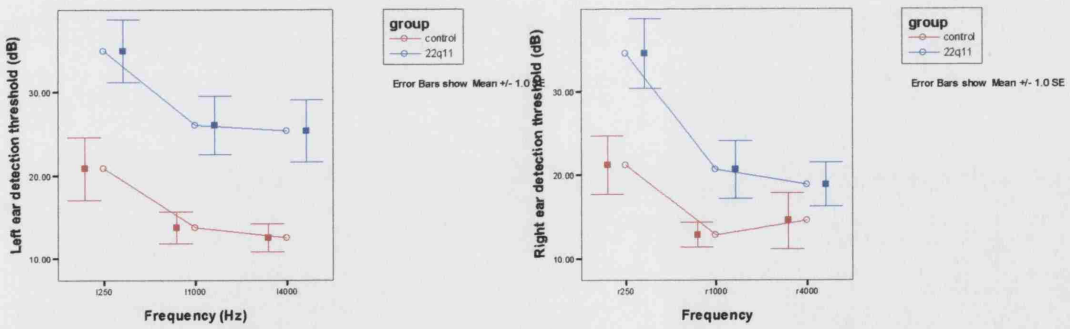
The group difference in hearing thresholds was surprising given that no participants reported current problems with hearing in everyday situations. However there is a high frequency of otitis media in children with 22q11DS, therefore fluctuating conductive hearing loss would be expected in this population. Peripheral hearing loss cannot be ruled out as a causative factor for auditory neural processing abnormalities, phonological impairment and language deficits in 22q11DS. To assess this possibility, the 22q11DS group were split into two groups based on average pure tone detection threshold (average threshold >= 30dB: impaired group (n=6), average threshold <30dB: non-impaired group (n=8)). No differences were found between these two subgroups of the 22q11DS population on any of the following indices:- participant characteristics (age, IQ), auditory discrimination skill (total scores on tone or speech same-different task), language ability (quick test, formulating sentences, CCC) or key ERP measures (tone N1a, duration MMN at Fz or A2, speech MMN at Cz or double deviant P3a). There were no correlations within

the 22q11DS group between average pure tone threshold and any of these measures. Sounds presented during the auditory experiments were at least 30dB higher than any participant's hearing threshold. However, the potential impact of hearing loss (both current and developmental) on central auditory processing and language development cannot be ruled out without direct comparison between the 22q11DS group and a group with both an equivalent history of otitis media and equivalent current hearing levels.

Table 5.11 Pure tone hearing thresholds in 22q11DS and control groups

Tone frequency	**Tone detection threshold (dB)**			
	22q11DS: mean (s.d.)		Controls: mean (s.d.)	
	Left ear	Right ear	Left ear	Right ear
250Hz	35 (14)	35 (15)	21 (13)	21 (12)
1000Hz	26 (13)	21 (13)	14 (7)	13 (5)
4000Hz	25 (14)	19 (10)	13 (6)	15 (12)
Average tone detection threshold	27 (10)		16 (8)	

Figure 5.8 Audiograms for 22q11DS and control groups



KEY CONTROLS 22Q11DS

5.4.2 Behavioural data

The mean hit rates for tone discrimination were 65% (s.d. = 25%) for control subjects and 48% (s.d.=21%) in 22q11DS. These low levels of performance indicate that this was a difficult task, however both groups' performance differed

significantly from chance (chance hit rate being 0% - equal probability of correctly detecting a difference and falsely ascribing difference to a same pair). Performance was normally distributed in both groups, and was not correlated with age or IQ (whole sample Pearson's R(age) = -0.19, R(IQ)= 0.28). Independent-samples t-test revealed no significant difference between groups on this task (t=1.7, p=0.1).

5.4.3 N1 responses to standard tones

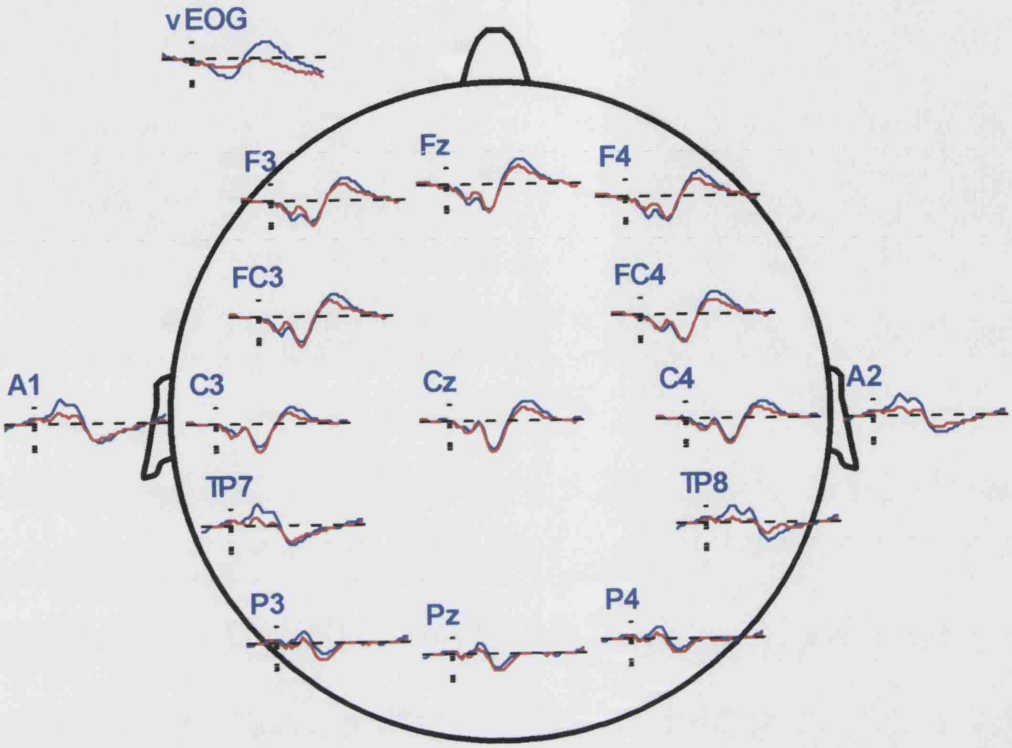
Visual examination of standard tone-elicited waveforms clearly indicated that the N1 complex at temporal electrodes was depressed in magnitude in the 22q11DS group relative to controls. GLM repeated measures ANOVA was conducted to explore group differences in the ERP responses to the standard tone entering data from both hemispheres and both early (N1a) and late (N1c) peaks. This confirmed a highly significant between-groups effect for the amplitude of responses (F[1,26]=12.4, p=0.002) and no significant group x hemisphere or group x component interactions. There was no consistent effect of group on the latency of either the early or late N1 potentials. The N1b component was detectable as a negative-going deflection within the large positive dip over the frontal electrodes in both groups, and no group difference was found for the trough-to-peak amplitude (F[1,29]=0.23, p=0.6).

interpretation? atypical process loss of tissue loss of functional organisation.

Table 5.12 N1 elicited by standard tone (22q11DS vs. controls)

Component	Electrode		Group	
			22q11DS	**Controls**
N1a	A2	amplitude	-1.3 (1.3)	-2.4 (0.9)
		latency	106 (18)	96 (13)
N1c		amplitude	-1.3 (1.2)	-2.4 (1.9)
		latency	169 (17)	164 (14)
N1a	A1	amplitude	-1.4 (0.8)	-2.6 (0.8)
		latency	104 (7)	104 (7)
N1c		amplitude	-1.2 (1.1)	-2.7 (2.1)
		latency	164 (16)	162 (14)

Figure 5.9 ERP waveforms elicited by standard tone (22q11DS vs controls)



KEY CONTROLS 22Q11DS

5.4.4 MMN responses to deviant tones

Visual examination of group mean ERP and subtraction waveforms (Figures 5.10 and 5.11) clearly indicated a discrepant pattern of deviant-related ERPs in the 22q11DS group. For all three deviants, responses at frontal electrodes were smaller in the 22q11DS group than in controls, whereas at temporal electrodes there was no difference between the magnitude or latency of responses.

A repeated measures ANOVA entering peak amplitude of the MMN subtraction waveform at electrode Fz for all three deviants, revealed a significant main effect of group ($F[1,28]=6.4$, $p=0.017$) and no group x stimulus interaction ($F[2,51]=0.7$, $p=0.49$). There was also a main effect of stimulus ($F[2,49] = 11.1$, $p<0.001$) indicating that both groups elicited larger responses for the double deviant than the single duration or frequency deviants. A similar repeated measures analysis for peak amplitudes of the mismatch response to all three deviants at electrode A2 revealed no effect of group ($F[1,28]=2.3$, $p=0.14$), nor a group x stimulus interaction ($F[2,47]=0.21$, $p=0.8$). Because of the observed group difference in N1 responses to the standard, a second set of repeated measures analyses were conducted entering N1a amplitude at the right mastoid as a covariate, to determine whether this could account for the group difference in MMN. However this strengthened the effect of group on frontal MMN ($F[1,26]=15.4$, $p=0.001$) and reduced the trend towards group difference in temporal MMN ($F[1,27]=1.4$, $p=0.25$). To confirm the consistency of MMN deficits across frontal electrodes in the 22q11DS group, duration MMN amplitudes were measured at additional electrodes (F3, F4, Fc3 and Fc4) and entered into repeated measures ANOVA. This confirmed the main effect of group ($F[1,29]=5.6$, $p=0.024$) and the enhancement of the effect by co-varying for N1 amplitude ($F[1,28]=13.8$, $p=0.001$), with no interactions between group and electrode in either analysis.

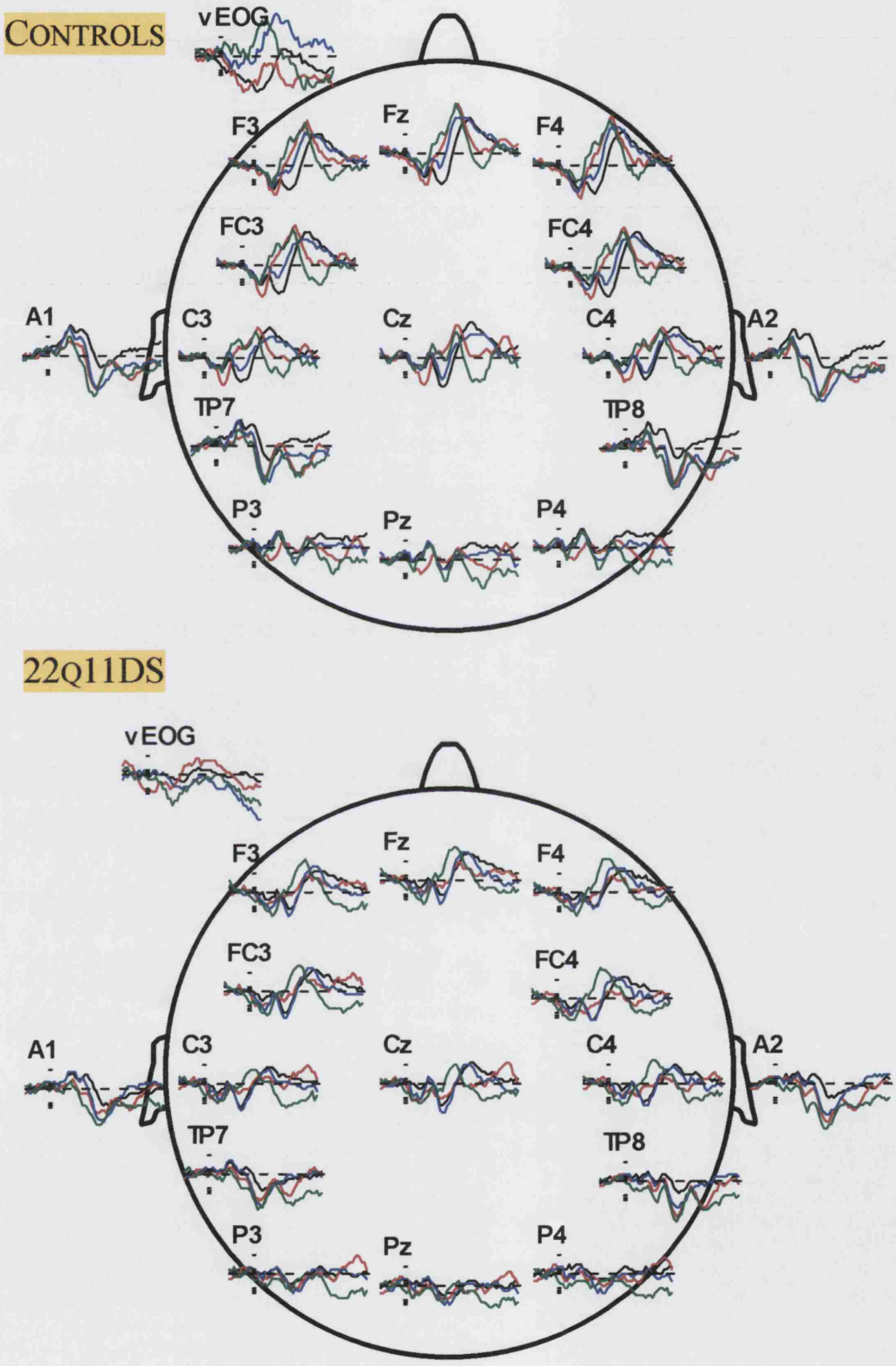
To confirm the contrast in group differences in MMN at the temporal and frontal sites, another repeated measures ANOVA was conducted entering the area measurements for MMN at both Fz and A2 for each of the three deviants (peak amplitudes cannot be used here because of the different polarity of responses), co-varying for N1a peak amplitude. This confirmed the main effect of group

(F[1,27]=5.5, p=0.026) and indicated a significant group x electrode interaction (F[1,27]=5.2, p=0.03), with no additional interactions between group, electrode and stimulus.

Table 5.13 MMN elicited by deviant tones (22q11DS vs. controls)

Deviant	Electrode		Group	
			22q11DS	**Controls**
Duration	Fz	amplitude	-3.2 (3.2)	-6.2 (2.8)
		latency	180 (35)	189 (34)
		area	386 (221)	554 (212)
	A2	amplitude	3.9 (1.8)	5.4 (3.3)
		latency	173 (33)	178 (37)
		area	358 (186)	470 (292)
Frequency	Fz	amplitude	-2.5 (3.0)	-4.5 (2.5)
		latency	210 (41)	186 (40)
		area	365 (267)	393 (230)
	A2	amplitude	3.2 (2.7)	5.0 (3.1)
		latency	181 (51)	198 (41)
		area	350 (193)	423 (250)
Duration + Frequency	Fz	amplitude	-5.3 (4.1)	-7.5 (4.2)
		latency	196 (32)	189 (37)
		area	436 (263)	599 (340)
	A2	amplitude	5.0 (2.4)	5.9 (2.3)
		latency	165 (33)	169 (23)
		area	485 (211)	511 (227)

Figure 5.10 ERP waveforms elicited by tone deviants (22q11DS vs. controls)

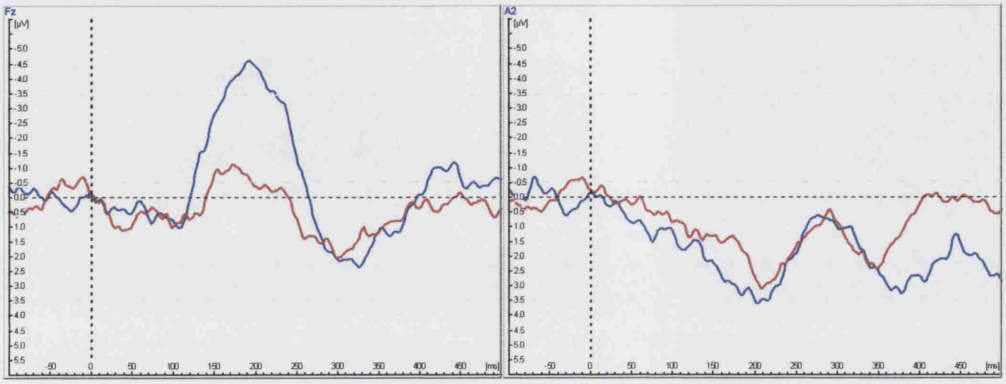


KEY STANDARD DURATION DEVIANT FREQUENCY DEVIANT
DURATION + FREQUENCY DEVIANT

Figure 5.11 Tone MMN (22q11DS vs controls)

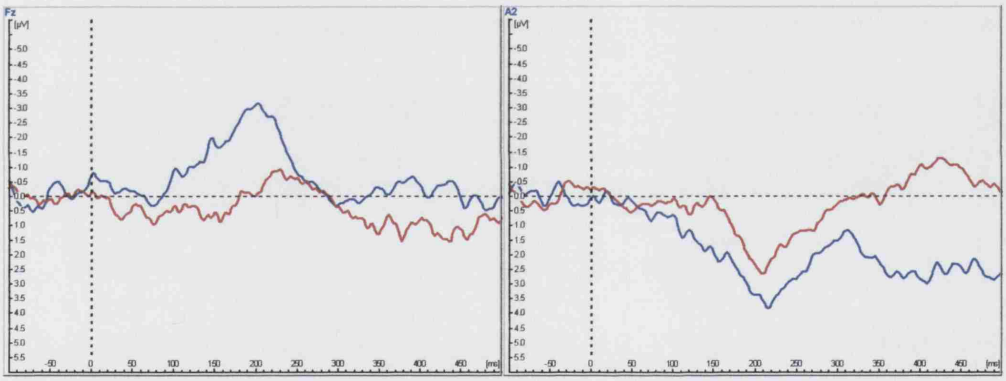
DEVIANT = DURATION

ELECTRODE FZ ELECTRODE A2



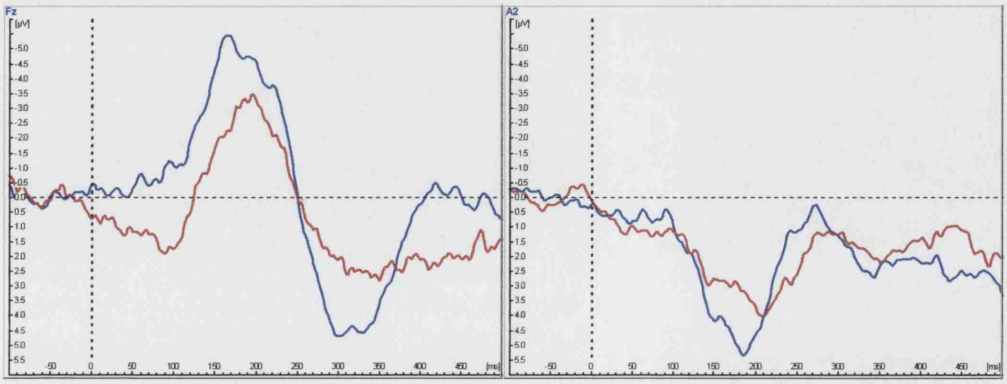
DEVIANT = FREQUENCY

ELECTRODE FZ ELECTRODE A2



DEVIANT = DURATION + FREQUENCY

ELECTRODE FZ ELECTRODE A2



KEY CONTROLS 22Q11DS

5.4.5 P3a responses to deviant tones

Repeated measures ANOVA entering P3a measurements (at electrode Cz) for all three deviants revealed no main effect of group for either amplitude ($F[1,28]=0.9$, $p=0.35$) or latency ($F[1,28]=2.1$, $p=0.16$), and no group x stimulus interactions. There was a significant effect of stimulus on P3a amplitude ($F[2,55]=10$, $p<0.001$) indicating that in both groups the double deviant elicited a larger response. There was a trend towards a significant group difference between P3a amplitudes for the double deviant ($t=1.7$, $p=0.09$), suggesting that the study may be underpowered to detect a group difference for this ERP component.

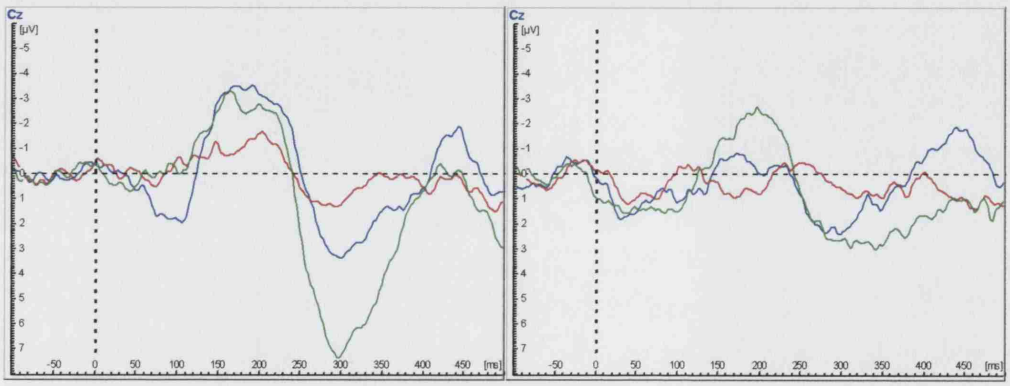
Table 5.14 P3a elicited by deviant tones (22q11DS vs. controls)

Deviant	Electrode = Cz	Group	
		22q11DS	Controls
Duration	amplitude	4.9 (3.6)	6.1 (5.3)
	latency	291 (41)	324 (36)
Frequency	amplitude	4.1 (3.2)	3.8 (3.3)
	latency	307 (45)	320 (59)
Duration + frequency	amplitude	5.9 (3.9)	9.3 (6.7)
	latency	310 (42)	324 (41)

Figure 5.12 Tone P3a (22q11Ds vs. controls)

ELECTRODE CZ

CONTROLS 22Q11DS



KEY DURATION DEVIANT FREQUENCY DEVIANT

DURATION + FREQUENCY DEVIANT

5.4.6 Change in tone ERPs across the testing block

Each subject's responses were reprocessed for analysis of responses recorded during the first and second halves of the testing block to determine whether the whole-sample response patterns and between-groups differences were fixed, emerged over time, or declined over the course of the testing session.

For both groups, N1 responses to the standard tone appeared stable at the temporal electrodes, but habituated at frontocentral sites. Repeated measures ANOVA revealed no effect of recording phase, and no phase x group interaction for N1a, whereas for N1b there was a highly significant reduction in amplitude across the two halves of the session ($F[1,29]=8.9$, $p=0.006$), with no interaction between recording phase and group.

The control group maintained stable MMN responses at both frontal and temporal electrodes to all three deviants throughout the session. However, response magnitude in the 22q11DS group at Fz appeared to increase from the first to the second half of the session (Figure 5.13). Entering MMN peak amplitudes at Fz from the first and second half of the testing block for all three deviants into repeated measures ANOVA confirmed the main between-groups effect ($F[1,28]=6.5$, $p=0.016$), indicated no main effect of testing phase ($F[1,28] = 0.5$, $p=0.5$) but a trend towards a group x phase interaction ($F[1,28]=2.6$, $p=0.1$). Post-hoc analysis indicated that the two groups differed significantly in the magnitude of frontal MMN elicited by duration and duration + frequency deviants during the first half of the testing block (duration $t=-3.9$, $p=0.001$; duration + frequency $t= -2.3$, $p=0.028$) whereas by the second half of the block these differences were not significant (duration $t=-1.7$, $p=0.1$; duration + frequency $t=-0.5$, $p=0.6$). The responses to the frequency deviant analysed separately for the first and second halves of the testing session did not reveal any significant group differences (first half $t=-0.9$, $p=0.4$; second-half $t=-1.1$, $p=0.3$), probably due to poor signal-to-noise ratio for this smaller response.

In contrast to this pattern of change in the frontal MMN across the testing session for the 22q11DS group, there were no similar patterns of change for MMN amplitude

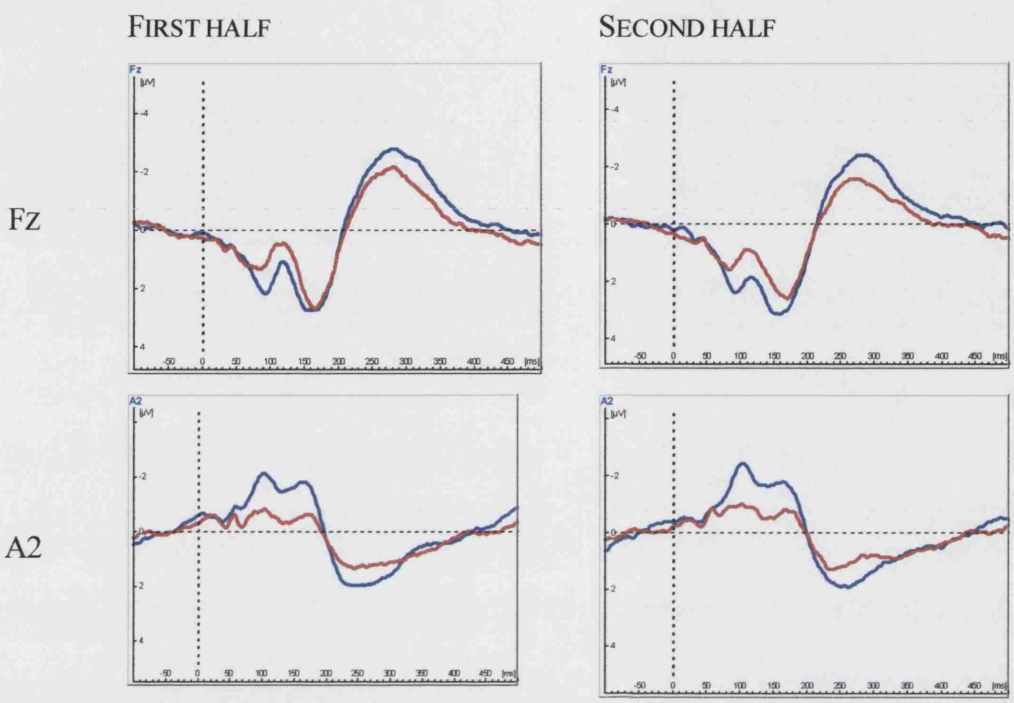
recorded at the right mastoid (main effect of phase F[1,28]=0.04, p= 0.8; group x phase interaction F[1,28]=0.2, p=0.6), or for the P3a component (main effect of phase F[1,28]=0.9, p=0.4; group x phase interaction F[1,28]=0.6, p=0.4). Split-half analyses confirmed the lack of between-groups differences for these ERP components (MMN at A2 F[1,28]=3.0, p=0.1; P3a at Cz F[1,28]=0.8, p=0.37).

Table 5.15 Change in tone ERPs across the testing block

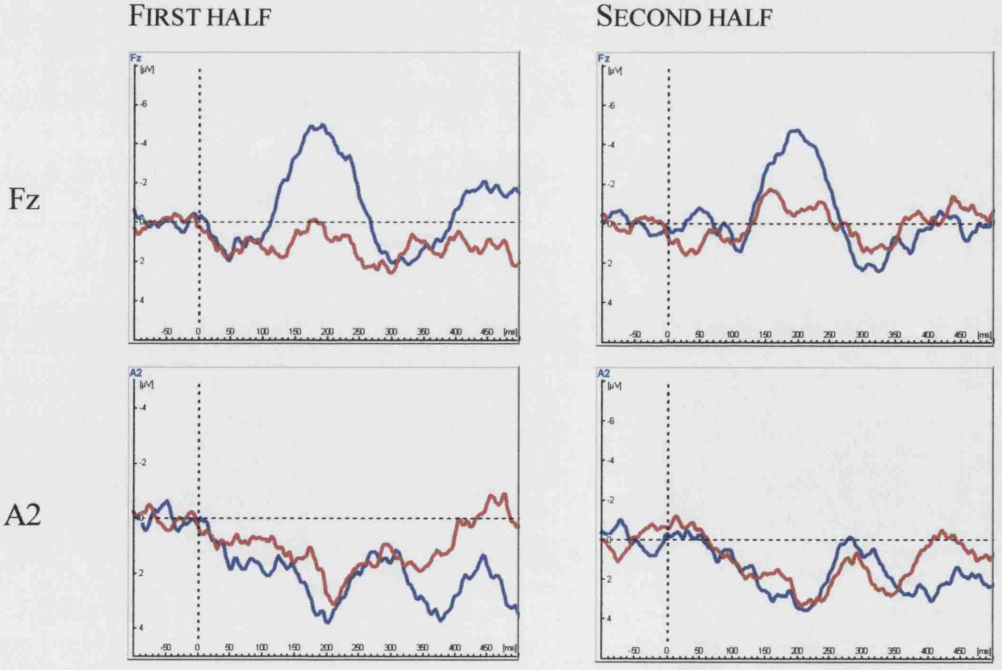
Component	Electrode	Stimulus	Block half	Group	
				22q11DS	Controls
N1a	A2	Tone standard	1	-1.2 (1.5)	-2.4 (1.3)
			2	-1.5 (1.1)	-2.6 (1.1)
N1b	Fz		1	-1.6 (1.1)	-1.6 (1.1)
			2	-1.2 (1.1)	-1.0 (0.8)
MMN	Fz	Duration deviant	1	-3.1 (3.4)	-7.4 (3.2)
			2	-4.5 (2.5)	-7.1 (4.6)
		Frequency deviant	1	-3.8 (4.1)	-5.1 (3.0)
			2	-4.0 (3.4)	-5.8 (3.5)
		Dur + freq deviant	1	-5.0 (4.1)	-8.7 (4.8)
			2	-6.8 (3.8)	-7.6 (5.1)
	A2	Duration deviant	1	3.7 (2.3)	5.5 (3.4)
			2	4.6 (2.5)	6.0 (4.6)
		Frequency deviant	1	4.4 (4.1)	4.8 (3.2)
			2	2.6 (3.2)	4.4 (4.1)
		Dur + freq deviant	1	6.1 (3.4)	6.6 (2.6)
			2	6.2 (4.1)	6.9 (3.3)
P3a	Cz	Duration deviant	1	6.3 (3.4)	7.1 (6.7)
			2	6.0 (5.0)	7.0 (5.1)
		Frequency deviant	1	5.1 (4.3)	5.2 (4.2)
			2	4.1 (4.8)	5.0 (4.4)
		Dur + freq deviant	1	7.9 (4.5)	9.4 (7.0)
			2	5.9 (4.8)	9.8 (7.9)

Figure 5.13 Change in tone ERPs across the testing session

TONE N1

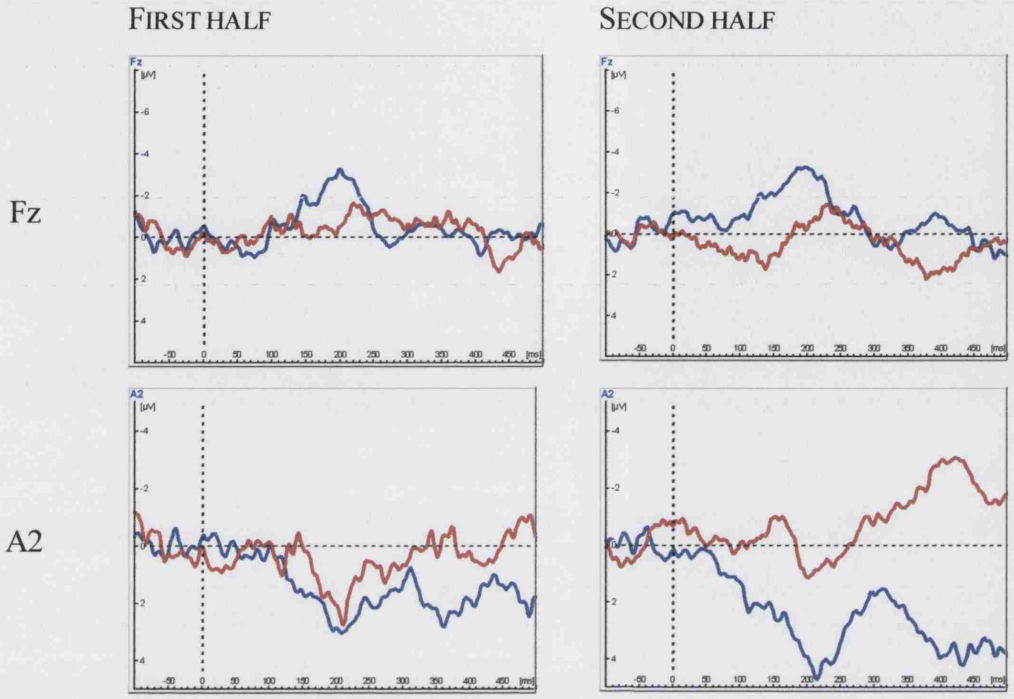


DURATION MMN

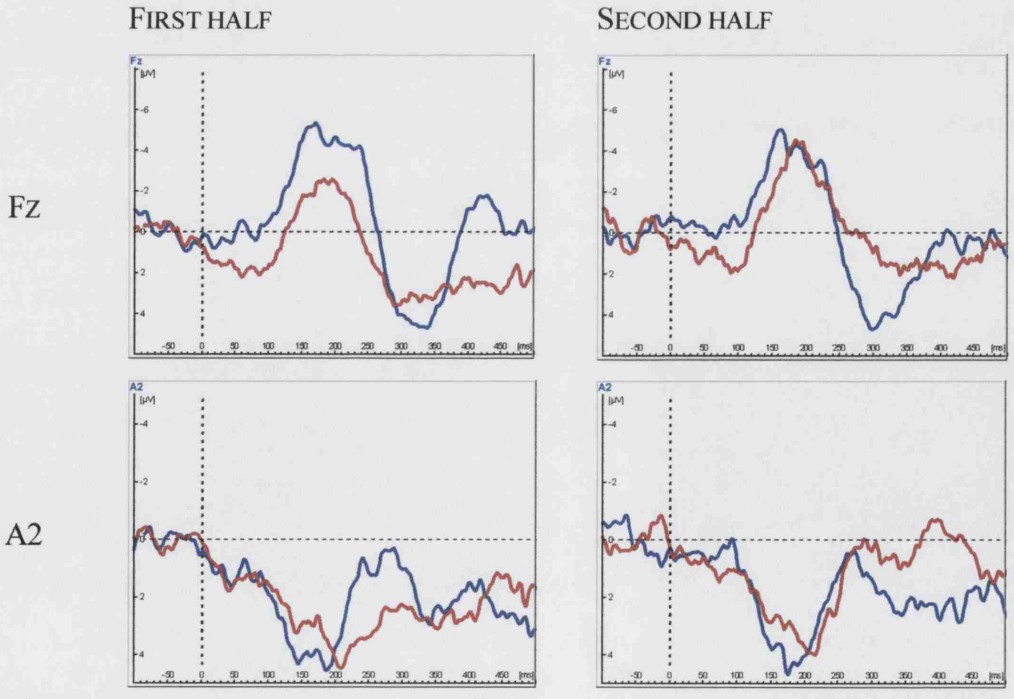


KEY CONTROLS 22Q11DS

FREQUENCY MMN



DURATION + FREQUENCY MMN

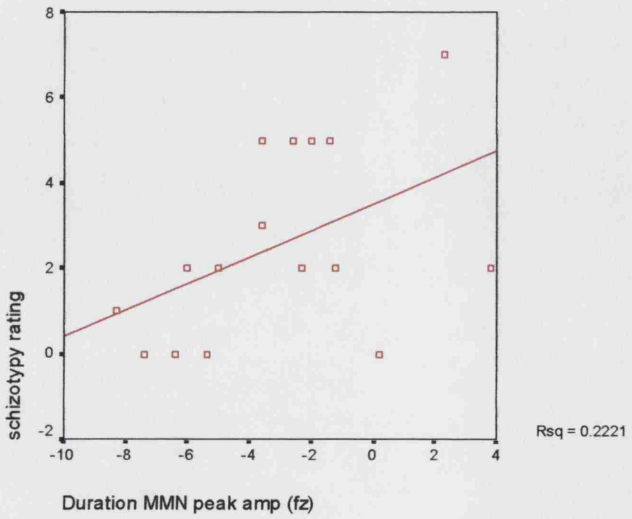


KEY CONTROLS 22Q11DS

5.4.7 Relationship between auditory ERP abnormalities and psychopathology in 22q11DS

Spearman's non-parametric correlations were computed between the ERP measure showing greatest difference between 22q11DS and control groups (duration MMN recorded at Fz) and two indices of psychological function hypothetically associated with psychosis-risk (the schizotypy scale and premorbid adjustment scale). In contrast to the working memory scale derived and tested for association with psychological risk in Chapter 4, the MMN measure is not correlated with IQ (22q11DS R=-0.2, p=0.6; controls R=0.4, p=0.2) therefore partial correlations are not necessary. An associated was detected between small frontal MMN and high scores on the schizotypy scale in the 22q11DS group which was statistically significant for duration MMN recorded during the first half of the testing block (R=0.48, one-tailed p=0.03), and approached significance for duration MMN for the whole testing block (R=0.32, one-tailed p=0.1). However no similar association was seen between the MMN measure and premorbid adjustment (first half R=0.04, p=0.5; total block R=-0.26, p=0.2). Examination of the scatterplot for schizotypy ratings and duration MMN (Figure 5.14) suggested that the relationship could be driven by one subject with the highest schizotypy rating and small MMN. However removal of this subject did not markedly change the strength of the correlation (R=0.4, p=0.07). No similar relationships were detected between schizotypy scores and N1a amplitude (R=0.12, p=0.3) or P3a (double deviant) amplitude (R=-0.2, p=0.2).

Figure 5.14 Relationship between duration MMN and schizotypy in 22q11DS



5.5 *Discussion*

5.5.1 Summary

- 22q11DS subjects displayed several atypical features of central auditory processing – diminished responses to the standard (N1 complex) at temporal electrodes and diminished amplitude of comparison-based responses to the deviants (MMN) at frontal electrodes. However the amplitude and latency of the P3a, attention-related, component did not differ between groups.
- The MMN impairment extends across tone contrasts in duration and frequency. The single-feature deviants revealed a more marked difference between groups than the double deviant contrast.
- The group difference in the magnitude of responses to the standard tones (the temporal N1 complex) did not account for the MMN deficit (since analysing MMN with N1 amplitude as a covariate increased the strength of between-groups effects).
- The frontal MMN deficit showed a pattern of change across the testing block. The groups only differed significantly in their response magnitudes during the first half of the testing session, and there was a significant increase in the responses within-subject between the first and second halves of the block in the 22q11DS group.
- The degree of frontal MMN deficit was associated with likelihood of demonstrating schizotypal personality features within the 22q11DS group.

5.5.2 Limitations

- Small sample size renders these results open to error, given that electrophysiological measures may be hampered by high signal-to-noise ratio. However the test-rest study indicated that N1 and duration MMN are stable phenomena within individuals over a period of several months. Between-groups effects were strong, and examination of individual waveforms and of individual quantified measurements indicated that results were not driven by clear outliers.
- The smaller responses elicited in both groups for the frequency deviant may render this group difference particularly open to error. Although group

differences were seen for this condition, frequency MMN as measured here may not be a reliable measure at the individual subject level.

- Whilst there was a striking dissociation between intact responses at the temporal electrodes and diminished MMN at Fz, little can be inferred regarding the cortical location of abnormal ERP generation. Concomitant structural and functional MRI data, that can be correlated with or even directly overlaid with ERP traces, would address this question and will hopefully be collected in the future.

5.5.3 Implications

This study has replicated and extended a previous report of central auditory processing disruption in 22q11DS using MMN as a marker of specific dysfunction (Cheour et al, 1997). It has been demonstrated that this neural processing abnormality is still observable when comparing 22q11DS individuals to individuals of equivalent general cognitive ability (i.e. mild / moderate learning disability), and that deficits are not limited to school-aged 22q11DS children. The previous study claimed that long inter-stimulus intervals were necessary to observe the MMN deficit in 22q11DS, however this is not the case, at least in adolescents, since groups clearly differed when a short ISI was employed.

Several aspects of the MMN deficit seen in 22q11DS subjects in this study are directly comparable to schizophrenia-associated deficits. The results are consistent with a growing number of reports (Baldeweg et al., 2002; Sato et al., 2003) indicating topographical specificity of MMN deficits in schizophrenia. Previous studies that did not report a dissociation between intact MMN at temporal electrodes and impaired frontal MMN may have been hampered by the use of either whole-head average reference or linked mastoid reference which distorts or eradicates the temporal component. The implications of this dissociation are that the MMN is not a unitary process but has several distinct neural generators. They also have different maturational course and different stimulus-magnitude sensitivity. However, further work is required to confirm the neuroanatomical basis for the dissociation, to establish whether the components are involved in distinct cognitive processes, and to determine whether there is any functional relationship between the two neural

systems, indexed by the different MMN components, which may be of relevance to psychosis.

The stimulus-sensitivity reported here is also broadly similar to that reported in schizophrenia. Javitt et al (1998) showed that although overall amplitude of responses to pitch change were diminished in the patient group, they were equally sensitive to the magnitude of the standard-deviant contrasts, as has been observed in this study. Secondly, equivalent diminution of MMN elicited by duration and frequency deviants, with a slightly more marked effect for the former contrast, is consistent with previous reports (Michie et al., 2000b).

Reduced N1 amplitudes have been inconsistently reported in the schizophrenia literature. Many studies did not report raw standard and deviant waveforms, only subtraction MMN, therefore N1 comparisons cannot be made. Gallinat et al (2002), employing different methodology (continually varying pitch and inter-stimulus interval, and requiring active response to tones) demonstrated N1 reductions, with a notable dissociation between frontal (impaired) and temporal (intact) responses. However this N1 may not be functionally or anatomically equivalent to that reported here. Small N1 could reflect either inefficient event-registration in the auditory cortex, or inefficient inhibition in a neural substrate acting to prevent transmission of irrelevant information. Further investigation of the dynamic properties of N1 and its relationship to MMN in both typical and atypical populations may clarify this issue.

The lack of a significant P3a deficit directly following reduced MMN in 22q11DS indicates that these ERP components reflect at least partially independent systems. Since P3a was found not to show any developmental change within the normal sample tested here, the dissociation between intact and impaired ERPs may indicate that later-maturing systems are more at risk in 22q11DS. Testing of more 22q11DS subjects over a wider age range is necessary to confirm this dissociation and explore developmental effects. The lack of group difference does not necessarily indicate that P3a indexes totally normal pre-attentive processing in 22q11DS. Disorganised transfer of information, indexed by a lack of coupling of P3a to MMN, could reflect inefficient information filtering or gating in 22q11DS, which has been theoretically and experimentally associated with psychosis in other populations and using other paradigms such as pre-pulse inhibition and suppression of the P50 ERP.

The observed within-subject changes over time in the magnitude of duration and duration + frequency MMN deficits seen in the 22q11DS population, with convergence towards control ERPs by the second half of the testing block, further extends our description of auditory processing deficits in this group. Baldeweg et al (1999b) showed that frontal MMN increases over time in normal adult listeners, using a paradigm in which the standard stimulus is continually changing. This procedure may stress the change-detection system such that a longer period of exposure to the stimuli is required before detection of deviants is reliable (and MMN emerges). Thus the slow increase in MMN, relative to stable responses in controls across the two halves of the block, indicates that there is no fixed lesion to the MMN system in 22q11DS, but that the parameters determining the speed at which the system establishes its steady state are altered.

Baldeweg et al (submitted) have also shown that, in normal subjects, as frontal MMN increases as a function of deviant-probability, N1 responses to the standard decline, indicating an adaptive process by which processing of the standard is inhibited and change-detection is enhanced. This paired-change in ERP elicitation in response to changes in deviant-probability is not seen in schizophrenia. The N1 reduction and dynamic MMN impairments seen in 22q11DS subjects may indicate that this series of adaptive changes are disturbed, such that more stimulus presentations or time are required in order to facilitate the establishment of reliable frontal MMN. A future task is to establish what experimental parameters change the efficiency of MMN emergence in both normal subjects and 22q11DS, and to determine what neurobiological parameters modulate this speed between individuals.

The relationship between frontal MMN and schizotypy scores within the 22q11DS group was predicted, but is still remarkable, especially given the small sample size tested here. This association confirms the relevance of the neurocognitive circuit indexed via this ERP as a marker of neuropsychiatric pathology. Follow-up testing of these subjects is required to determine whether ERP characteristics change in the face of fluctuations in mental experience over time, or whether the association reflects a stable, developmentally-emergent link between neurocognitive abnormality and relative neuropsychiatric risk within the 22q11DS group.

In summary, it has been demonstrated here that 22q11DS adolescents display reduced MMN at frontal electrodes, similar to that seen in schizophrenia. Significant P3a abnormalities were not observed, although they are commonly reported in schizophrenia, suggesting that this later component may become disrupted over time, in association with the emergence of symptoms or cognitive decline. Alternatively the contrast between observations in 22q11DS and in schizophrenia may indicate that the neurocognitive pathways towards psychosis in the syndrome and in the idiopathic situation are in some respects distinct. The pattern of ERP deficits reported here for 22q11DS is consistent with that reported by Michie et al (2002), who found that relatives of schizophrenia patients showed MMN reductions equivalent to those seen in patients but did not display P3a reductions (which were seen in the patient group). Hence MMN, but not P3a, abnormalities may reflect genetic risk for psychosis and may be valid endophenotypes for further study in 22q11DS and in other high-risk populations.

6 Speech processing - a potential neurophysiological endophenotype for psychosis in 22q11DS

6.1 Introduction

6.1.1 Overview

In selecting candidate endophenotypes for investigation as potential markers of psychosis in 22q11DS, we were guided by existing literature both on neurodevelopment and schizophrenia, and atypical developmental features in 22q11DS. Review of both literatures, and preliminary psychiatric / cognitive assessments of 22q11DS subjects, indicated that language and communication was a potentially significant area of shared developmental difficulty. Language deficits are a component of the schizophrenia phenotype, and language delays and deficits have been observed in several high-risk studies. Speech and language impairment is a predominant component of the 22q11DS phenotype, with communication difficulties ranging from mild to very severe throughout childhood and adolescence, and in many cases resistant to amelioration by prolonged periods of therapy. Hence a specific, language-relevant, developmental pathway may be vulnerable to disruption in 22q11DS. Evidence for language-relevant developmental disruption was sought via neurophysiological investigation of speech processing using the same basic ERP methodology as previously described.

The status of a speech processing abnormality as a potential endophenotype for psychosis was assessed in the same manner as other cognitive and neural candidate markers, by examining the relationships between speech ERP measures and interview-derived schizotypy ratings. In addition, the potential impact of speech processing abnormalities on language function in 22q11DS was assessed by examining the relationship between ERP measures and behavioural measures of language performance.

6.1.2 Language development in typical and atypical groups

There are many factors that could potentially constrain a child's ability to learn to understand and produce speech, and to develop skills necessary for complex communication. At the simplest level of description, these potential constraints on development can be divided into input constraints (peripheral and central auditory processing), output constraints (central and peripheral motor sequencing and execution of articulatory gestures) and constraints in what could be termed the central "black box", i.e. the developmental mapping processes by which the input and output systems are connected, and integrated with other cognitive processes. In contrast to the input and output systems, knowledge regarding the brain mechanisms for language development, including mapping between production and perception, is largely speculative at present. Investigation of the emergent speech and language skills in atypical populations can add to our understanding of language development, by observing the impact of distortions in the strength and weakness of interacting systems that are involved in the developmental process.

Input constraints clearly impact on speech development and language comprehension in children with moderate hearing impairment (Briscoe, Bishop, & Norbury, 2001). Similarly "output" constraints, for example stuttering and speech ataxias, have been found to be associated with structural abnormalities in perceptual and motor speech areas in the brain (Kent, 2000). A particularly striking demonstration of the relationships between speech production and higher-level language ability is provided by a family with an inherited oromotor dyspraxia (the KE family) investigated by Vargha-Khadem and colleagues. The affected members of this family carry a mutation in a single gene, FOXP2, a transcription factor on chromosome X (Lai, Fisher, Hurst, Vargha-Khadem, & Monaco, 2001). Individuals carrying this mutation have severe speech production impairments (Watkins, Dronkers, & Vargha-Khadem, 2002), volumetric changes in the basal ganglia and other cortical regions (Belton, Salmond, Watkins, Vargha-Khadem, & Gadian, 2003), and pervasive difficulties with comprehension, communication and literacy in the face of normal non-verbal intelligence. It is currently unclear whether the articulatory and higher level language impairments are causally linked, or the result of parallel disruptions effected by the FOXP2 mutation.

Central (brain) mechanisms clearly play a part in mediating language development in atypical groups with either input or output constraints, but little is known at present about what these central mechanisms might be. The extent of interaction between peripheral and central mechanisms seems likely to be widespread and fundamental in atypical populations, although it is not yet possible to determine whether brain changes are causative or consequential, and whether they relate in a direct fashion to developmental interactions within the typically-developing population. The development of language organisation in the brain is a highly interactive process involving multiple sources of neural activity, and diverse influences that will include both genetic and environmental factors. At every stage, language-related activity (both productive and perceptual) will feed-forward into the organisation of language-relevant brain networks, which in turn will guide behaviour in a manner that may influence the next stage of development (Kuhl, 2000). Establishing neural markers for components of language organisation in the brain could facilitate future studies of developmental interactions between factors, within different populations.

6.1.3 Speech and language development in 22q11 Deletion Syndrome

22q11 Deletion Syndrome is frequently associated with laryngeal abnormalities that cause some degree of structural compromise of speech production mechanisms. According to a large survey of clinical case notes (Ryan et al., 1997) palate anomalies were present in 14% of 496 patients (9% overt cleft, 5% submucous cleft) and velopharyngeal insufficiency in a further 32%. In addition, variable levels of conductive hearing impairment often accompany palate anomalies because of the shared embryological origins of the ear and palate structures. For many individuals with 22q11DS, impairments in speech production continue into late childhood or beyond even following surgery and intensive speech and language therapy and in the face of normal hearing. These production impairments vary from functionally non-intrusive hypernasality, to severe impairments in speech intelligibility. The cause of these persistent production impairments is unclear. However, peripheral constraints on speech production and / or hearing impairments do not appear to account for the full spectrum of language disorders in the syndrome.

In one of the very few systematic studies published to date, children with 22q11DS were found to display more severe speech production and comprehension deficits than other children with equivalent palate malformations and velopharyngeal dysfunction (and therefore similar levels of risk for conductive hearing loss) who had been assessed and treated at the same clinical centre (Scherer, D'Antonio, & Kalbfleisch, 1999b). This suggests that additional developmental mechanisms differentiated the 22q11DS group from the control population. Little is known at present as to the consequences of early or prolonged difficulties in speech production for the development of speech comprehension and language skills in 22q11DS. However, a small number of 22q11DS cases have been reported in which the onset of speech and language development has been severely delayed in the absence of either articulation deficit or general learning disability (Goorhuis-Brouwer, Dikkers, Robinson, & Kerstjens-Frederikse, 2003). This suggests that neither structural anomaly nor non-specific cognitive impairment are sufficient to explain language impairments in 22q11DS, and that some additional factor(s) may be important. Hypothetically, genetically-mediated disruption of a neural processing mechanism, necessary for the development of some fundamental aspect of language, may be responsible for both prolonged speech production deficits, comprehension and communication difficulties in some individuals with 22q11DS.

A systematic study of the speech output characteristics of 21 children with 22q11DS was carried out by D'Antonio, Scherer, Miller, Kalbfleisch, & Bartley (2001). This group reported that, by age 7, consonant repertoires contained the full range of manner of articulation (defined according to the manner in which the air stream is modulated by the articulators) and place of articulation (defined according to the location within the vocal tract of the primary modulation of the air stream). However, there was an unusual preference for the use of "voiceless" rather than "voiced" consonants. Voicing refers to the presence or absence of vibration of the vocal folds during consonant production, and is a vital acoustic cue (for example, the addition of voicing to the consonant /t/ converts the signal to /d/). Voicing requires additional motor support of the air stream, and neural control of the vocal chords in co-ordination with the upper articulators within the pharynx. The delay or deficit in voicing in 22q11DS, reported by D'Antonio et al, could come about because of neural, muscular or respiratory compromise, but is unlikely to be dependent solely on the structural integrity of the palate. There is no published research evidence to

suggest that this atypical pattern of consonant production is reflected in corresponding perceptual deficits, but this is a testable hypothesis. Insensitivity to specific phonetic cues in the speech stream, arising as a consequence of or alongside atypical speech production patterns, could potentially hinder receptive and expressive language development. If such insensitivity exists, it should be detectable by neurophysiological means.

6.1.4 Speech and language as a component of risk for schizophrenia

A central role for language disorder in risk for schizophrenia and in the psychopathology of the illness itself has long been discussed. Language abnormalities form a major component of the diagnostic criteria, since the phenomena contributing to the symptom dimension of positive thought disorder describe abnormalities in language. These phenomena include bizarre or tangential use of speech, neologisms and loosening of associations. Linguistic and communication deficits are also recognised as part of the negative syndrome, in the form of poverty of speech and social withdrawal. The presumption that these deficits are epiphenomena of disruptions in "thought" has been challenged by clinical investigators, notably Andreasen (1979), who constructed a scale to measure thought, language and communication impairment, in order to demonstrate that these abnormalities were a symptom dimension in schizophrenia, independent of other positive or negative features.

Like other aspects of schizophrenia, language can be studied from many different perspectives and at different levels of analysis. The most challenging theoretical viewpoints on the significance of language disorder in schizophrenia have been put forward by investigators suggesting that disruption in the relationship between thought and language lies at the heart of psychosis. Crow has long argued that schizophrenia arose as an evolutionary by-product of the emergence of language in homo sapiens (Crow, 2000), however evidence for this view has been weak. A more cognitive proposal (Maher, 2003) is that disconnection between thought and utterance would result in delusions of control, an extension of the view of Frith that disconnection between willed action and motor feedback (aberrant self-monitoring) would result in the formation of delusional explanatory frameworks (Frith et al.,

2000). A speech-monitoring deficit could account for diverse symptoms including auditory hallucinations (misattribution of perceived utterance to self or others) and anomalous expressive language function.

There is now considerable functional imaging evidence from both MRI and ERP studies that abnormal activity in language regions in the brain is associated with psychotic symptoms. Specifically, auditory verbal hallucinations are associated with uncontrolled activity in the superior temporal lobes, whilst hallucinations in other modalities such as somatosensory illusions are associated with activity in corresponding sensory areas (Shergill et al., 2001). The association between abnormal perceptual experiences and aberrant activity in specific neural areas has long been presumed given the relationship between epilepsy, in particular temporal lobe epilepsy, and hallucination-like experiences (differentiated from true hallucinations because they are not generally accompanied by the strength of conviction that the sensations are "real"). However it is not surprising that when one "hears voices" one activates the "voice-hearing" regions of the brain, and a causal connection between disruption in speech processing centres and risk for hallucinations has yet to be demonstrated. Addressing the self-monitoring hypothesis directly, Ford and colleagues showed that individuals who experience hallucinations fail to display the normal suppression of an N1-like ERP when hearing one's own voice relative to the sound of another speaker (Ford et al., 2001). This is weak but suggestive evidence for disorganisation of neural circuitry involved in speech processing as a predisposing vulnerability to psychosis.

An alternative neuropsychological explanation for the existence of language abnormalities in schizophrenia would be that, since language involves function of almost every area of the human brain, any neural disruption leading to increased risk of psychosis would be likely to result in language abnormalities. This would predict that as psychiatric symptoms fluctuate (because of changes in the degree of neural disruption), language disorder would also vary within the individual. This is seen for some aspects of language disorder, in some patients, for example the production of neologisms and tangential, uncontrolled speech during florid psychosis in contrast to poverty of speech production and content during remitted phases, when negative symptoms are more likely to predominate. Evidence for parallel changes in symptoms and language function was found by Schuepbach et al (2002), who

showed that remission of negative symptoms was accompanied by improvements in verbal fluency in first-episode patients. The co-occurrence of language disorder with symptoms, indicating dependence on a common set of neural substrates, could also contribute to a vicious cycle of social experience – individuals with a predisposition to both psychosis and communication impairment could be at especially high risk for social isolation and a chronic course of psychotic episodes and functional impairment.

Do language abnormalities correspond to the definition of an endophenotype for psychosis? Some components of language, such as general comprehension and use of complex linguistic constructs, may be stable reflections of neurobiological risk, as shown in a longitudinal study (Condray, van Kammen, Steinhauer, Kasparek, & Yao, 1995). However these factors are likely to be very non-specific. Some abnormalities (more linguistic errors) are shared with bipolar affective psychosis, whilst others (reduced complexity) appear to be limited to schizophrenia (Thomas et al., 1996), even once potential confounding factors such as social class, memory impairment and attention have been accounted for. Cohort studies have reported high levels of speech delay and language abnormality in individuals who go on to develop schizophrenia (Jones, Rodgers, Murray, & Marmot, 1994) and in their siblings (Bearden et al., 2000), although not all studies have collected sufficient information to support this claim.

Language abnormalities, similar to those categorised as thought disorder in adult patients, were reported for the New York high-risk sample. Thought and communication disorder was found during childhood and adolescence in offspring of schizophrenic, but not affective-disordered parents, and was predictive of schizophrenia diagnosis in adulthood as early as age 9. This confirms that language abnormalities reflecting neurodevelopmental risk for psychosis are present many years before the onset of illness (Ott, Roberts, Rock, Allen, & Erlenmeyer-Kimling, 2002). In childhood-onset schizophrenia, moderately severe speech and language abnormalities are seen both in patients and their well-siblings, suggesting that these abnormalities reflect shared genetic risk factors and not state-related expressions of the illness (Nicolson et al., 2000). Asarnow et al (2002) found that children with a weak family history of schizophrenia (affected second-degree relative) had poorer receptive and expressive language scores than controls without family history,

although they also displayed poorer general ability and neuromotor development, questioning the specificity of language impairments as specific risk indicators. Collectively these high-risk studies indicate that language abnormality is a component of the genetically-mediated neurodevelopmental disruption contributing to risk for psychosis.

Studies in adult twin pairs and adult siblings discordant for diagnosis have indicated a complex relationship between genetic relatedness, language function and psychiatric outcome. Condray, Steinhauer, & Goldstein (1992) found that brothers of schizophrenia patients displayed reduced language comprehension, but not reduced performance on tasks of general ability or executive function. Docherty and Gottesman (2000) found that communication ratings of speech samples obtained during semi-structured interviews discriminated between affected and unaffected twins, suggesting that language deficits in a more naturalistic setting are characteristic of illness-state and not genetic liability. An important distinction, therefore, must be drawn between measures of language behaviour (as assessed by communication ratings and some neuropsychological tests) and language-related neural functions. The latter may show endophenotype-type status, whilst the former probably does not.

6.1.5 Event-related potentials and speech processing

The processing of deviance within a stream of regular speech sounds elicits a sequence of ERPs that is very similar to that elicited by deviance in the simple physical features of sound (Kraus, McGee, Sharma, Carrell, & Nicol, 1992). However, speech MMN indicates pre-attentive detection of more than just acoustic deviance. It probes the language-relevant organisation of auditory information. Phillips et al (2000) presented sounds from a /da/-/ta/ voice-onset-time continuum that were matched on the magnitude of acoustic deviance but corresponded to either within-category or between-category phonological difference. This study showed that adult listeners only detect change at the level of the MMN when a contrast that is phonologically-relevant is presented (i.e. within-category deviance does not elicit an MMN). This observation has been extended in cross-language studies. Winkler et al. (1999b) presented Hungarian and Finnish listeners with two vowel pairs, each of which was a within-category contrast in one language whilst being a between-

category contrast in the other. MMN was elicited to both contrasts in all listeners (indicating that sufficiently large acoustic deviance is pre-attentively detected regardless of linguistic relevance) but for both groups of listeners, the between-category deviant elicited a larger MMN. Moreover, speech MMN has been shown to be plastic in the face of language experience. Speech MMN for non-native contrasts can be acquired by second-language learning, in both adults (Winkler et al., 1999a) and children (Cheour et al., 2002).

The auditory cortex is capable of extracting abstract relationships from highly variant acoustic cues, but it does so selectively, with the assistance of top-down influences that seem to emerge developmentally as a consequence of language experience. Responses to speech contrasts are modulated by context, in that responses increase when sounds are presented within words (and non-words) rather than occurring alone (Kujala et al., 2002). Thus speech MMN may be a valid probe for representations and processing relationships that are actually utilised during speech comprehension. Current theory suggests that the elicitation of speech MMN involves a matching process between an afferent acoustic signal and a memory representation corresponding to a linguistically-relevant relationship between sounds. However empirical contributions to this model are currently very limited.

Although observations regarding pre-attentive detection of speech contrasts indicate the sophistication of information processing that can be achieved by the auditory system, and the impact of language experience on these processing capabilities, they do not address the functional significance of these phenomena for language development or performance. These questions are more easily addressed via studies of atypical populations. Information regarding speech processing in atypical groups comes from two sources – adult aphasic patients and children with developmental speech and literacy disorders. Csepe, Osman-Sagi, Molnar, & Gosy (2001) showed that phonemic MMN was disrupted in four aphasic patients whereas pitch processing was intact, regardless of the types of aphasia (Broca's i.e. expressive or Wernicke's i.e. receptive). Moreover, it was demonstrated that it is possible to have a selective deficit in the ability to generate MMN to voicing or place of articulation contrasts, and that these selective neurophysiological deficits correspond well to each patients' behavioural deficits in speech sound discrimination. This confirms that phonetic

features are processed not only in speech-specialised neural circuits but that different cues in speech may be processed in distinct distributed circuits.

Groups with atypical language development have also been investigated with respect to differential deficits in speech ERPs but results have been somewhat ambiguous to date. Some have claimed speech-specific ERP deficits, firstly in children with specific language impairment (Uwer, Albrecht, & von Suchodoletz, 2002) and secondly in children with autism (Ceponiene et al., 2003). Others have demonstrated more basic perceptual processing abnormalities in SLI (Korpilahti & Lang, 1994), dyslexia (Baldeweg, Richardson, Watkins, Foale, & Gruzelier, 1999a) and autism (Gomot, Giard, Adrien, Barthelemy, & Bruneau, 2002). To conclude whether speech processing ability mediates a proportion of variance in normal language and literacy attainment, longitudinal studies of the impact of individual differences in speech representations on development are required.

6.1.6 Speech ERP studies in schizophrenia

In line with the concept that schizophrenia may arise, at least in part, as a consequence of quite low-level perceptual anomalies and neuronal disruption, and that an important set of perceptual and cognitive capabilities of relevance to schizophrenia involve speech and language processing, a small number of investigations of speech processing in schizophrenia have been carried out. Using classic behavioural tests of speech processing, schizophrenic subjects differed from controls in the "sharpness" of a categorical boundary between /ba/ and /da/ stimuli along a continuum (Cienfuegos, March, Shelley, & Javitt, 1999). This suggests that individuals with schizophrenia may have less "well-defined" memory representations for speech information, although this may be a consequence of a more generalised memory or representational deficit. This finding contrasts with the performance of other memory-impaired groups, e.g. Alzheimer's patients, in whom categorisation impairments are not generally found (Kurylo, Corkin, Allard, Zatorre, & Growdon, 1993). These behavioural findings have recently been extended to an EEG and MEG study (Kasai et al., 2002; Kasai et al., 2003) in which patients with schizophrenia were found to have more markedly impaired phonemic MMN than tone (duration) MMN. The MEG study indicated bilateral abnormalities in the auditory cortex during speech processing, whilst source-modelling using the EEG data implicated

additional neural dysfunction in the right frontal lobe, suggesting aberrant frontotemporal connectivity during speech processing in schizophrenia.

6.1.7 Summary

In 22q11DS, language development may play a part in mediating risk for psychosis, or may reflect disruption in psychosis-relevant neural circuits. Neural indicators of disrupted speech processing in 22q11DS could be endophenotypes for psychosis-risk and thus point towards causal mechanisms. Moreover, by comparing language-relevant processes in the brain between different atypically developing groups, and assessing within-group relationships between brain processes and behavioural outcomes, it is hoped that components of neural processing relevant to language development in the general population may be identified.

In order to carry out this investigation an experiment was designed to probe the neural organisation of speech perception. Because of the potential influence of articulation deficits on speech organisation in the 22q11DS group, and reports that phonetic relationships such as voicing and place of articulation may be independently processed in the brain, particular attention was paid to devising a method of identifying phonetic-acoustic associations in the neural processing of speech sounds.

6.2 Method

6.2.1 Experimental aims

This speech processing experiment was designed in parallel with the tone experiment described in Chapter 5. In both experiments, two parallel hypotheses were tested in a pilot experiment with normal adult subjects:

1. The size of MMN reflects the magnitude of the acoustic difference between the standard and the deviant and should therefore be larger when two or more features are combined within a deviant than when a single feature is presented alone. In speech, a "feature" corresponds to a change along a phonetic dimension i.e. voicing, place of articulation or manner of articulation.
2. Phonetic features may be processed by partially independent neural systems (as is the case for pitch, duration and intensity deviance). If so, responses to any individual speech stimulus should show context-dependence, according to the standard with which it is paired, which will determine the phonetic contrast between the standard and the deviant. To test this hypothesis a balanced design was employed, involving two different standard stimuli and three deviants, to access responses to phonetic deviation irrespective of the phonology of the stimulus.

A test-rest study was conducted as described in Chapter 5 to confirm both group-level results and individual stability of responses. Developmental data collected from control subjects in the 22q11DS and SLI experiments was combined with the adult pilot data to assess the maturational course of speech ERPs.

6.2.2 Experimental design

Both speech blocks contained the same three consonant-vowel stimuli as deviants (/ta/, /ga/, /ma/) but the standard stimulus differed between blocks (/da/, /ka/). Therefore although the absolute properties of the deviant stimuli are constant between blocks, the phonetic contrasts which separate the standard from each of the deviants are not constant, and six different standard-deviant pairings are created. Table 6.1 describes the phonetic contrasts generated as a consequence of the experimental design.

Testing procedures were identical to those described in Chapter 5 for the tone experiment. Speech and tone blocks were presented in random sequence both for the test and retest pilot experiments and for the case-control studies.

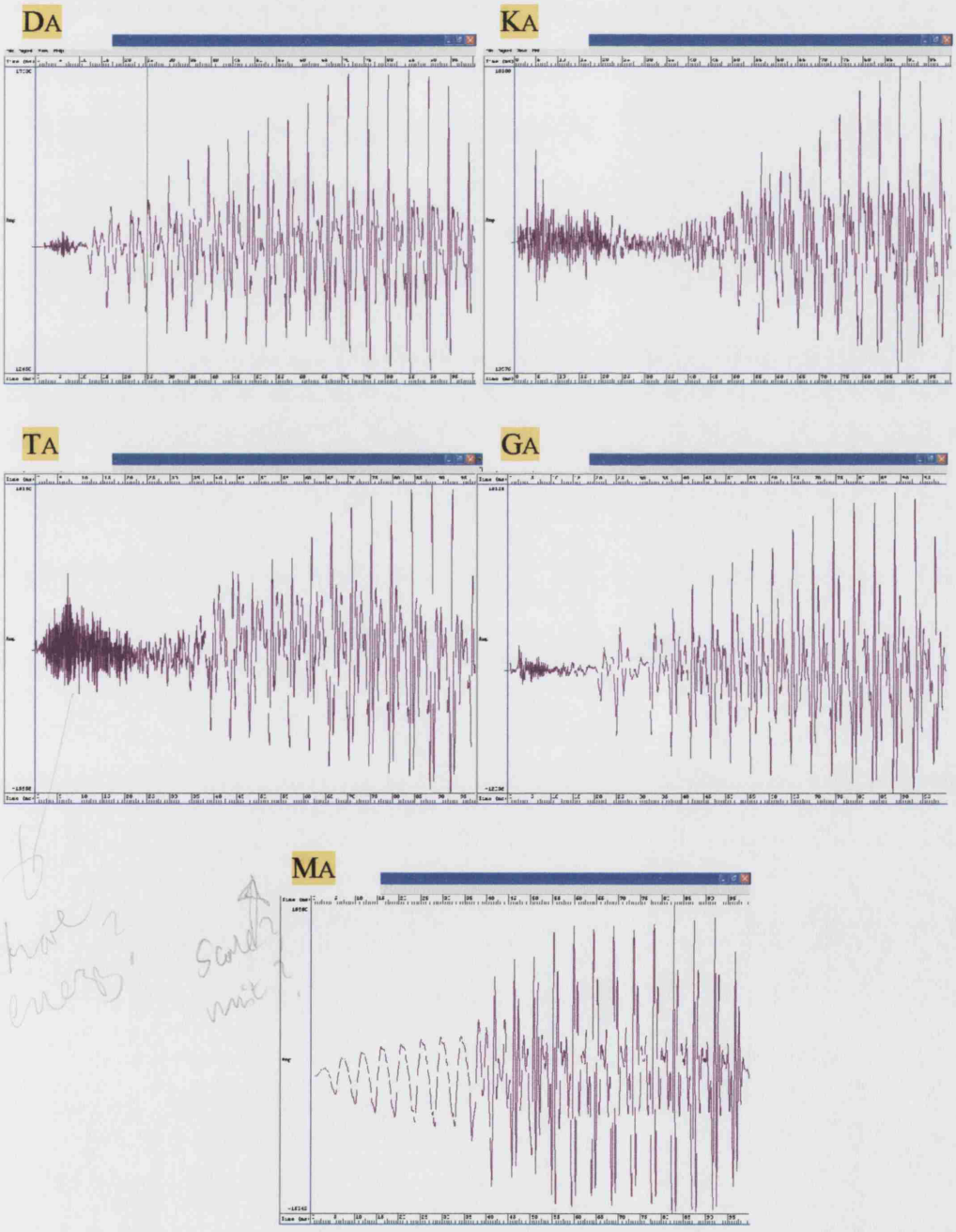
Table 6.1 Speech ERP experimental design

Phonetic contrast between standard and deviant		*Standard*	
		Speech block A	Speech block B
		da	*ka*
Deviant	*ta*	Voicing	Place of articulation
	ga	Place of articulation	Voicing
	ma	Place of articulation and manner of articulation	Voicing, place of articulation and manner of articulation

6.2.3 Stimuli

Natural speech stimuli were spoken by a female native English speaker and recorded via Cool Edit '96 on a PC fitted with Creative Soundblaster sound card. These were digitally matched for total stimulus duration (100ms), average intensity (RMS) and onset-offset amplitude window.

Figure 6.1 Spectrographs for speech stimuli



6.3 *Preliminary Results*

6.3.1 Pilot and reliability study

6.3.1.1 N1 responses to standard speech sounds

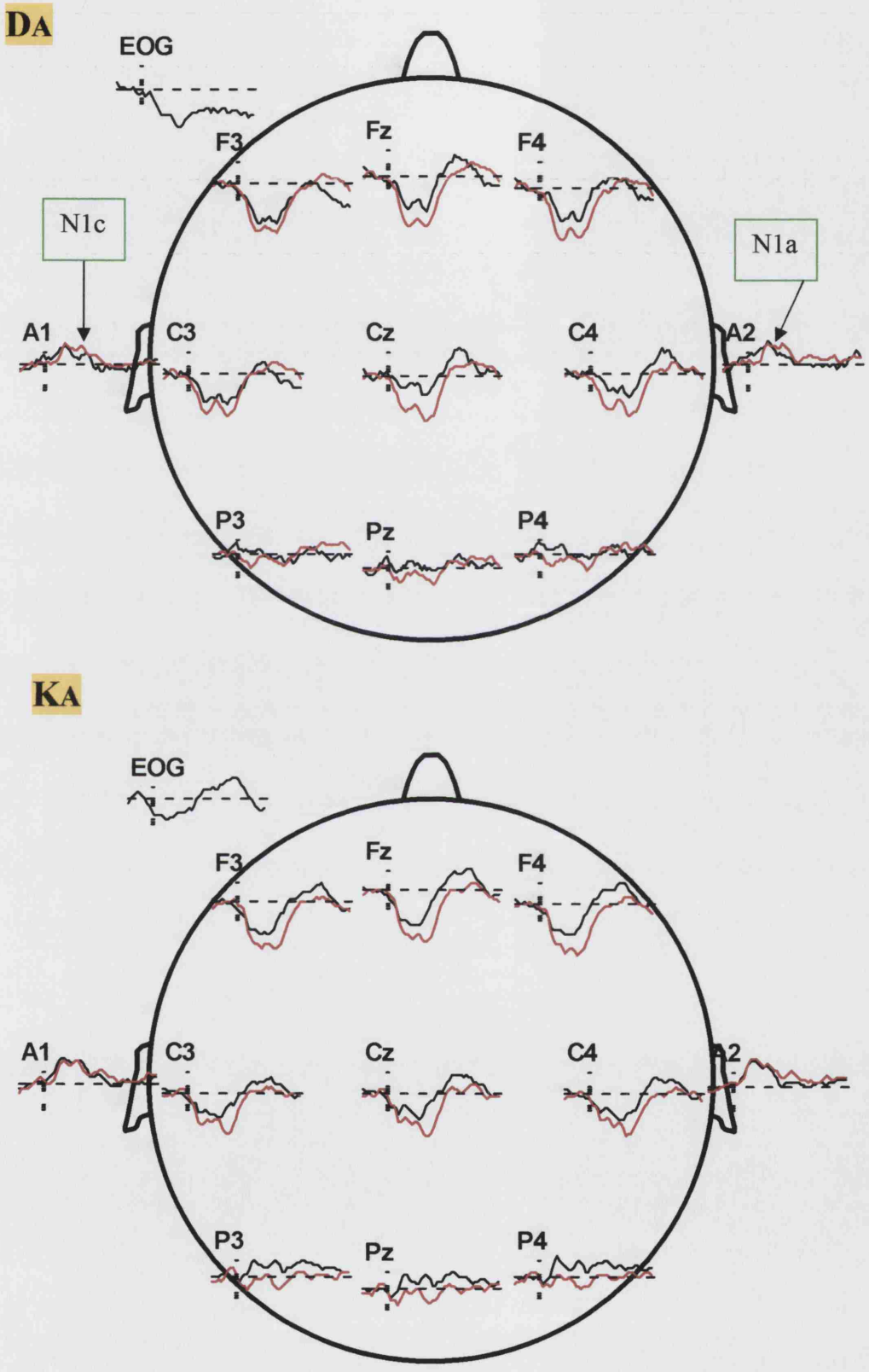
Both standard speech sounds generated a broad N1-like response over temporal electrodes peaking at around 100ms post stimulus-onset. For the /da/ stimulus only a single peak (N1a) was evident, whereas for /ka/ a second peak (N1c) was present at around 150ms on both the left and right sides. This is in line with the observation that voice-onset time characteristics influence the morphology of the N1 response (Sharma & Dorman, 1999). No frontocentral N1b component was evident for either speech standard, suggesting that spectrally complex stimuli do not activate tonotopically distinct areas of the auditory cortex. There were no group-level differences in the N1 responses at test and retest, and responses on the right-hand side were reliable at the individual level. Repeated measures ANOVA entering the amplitude of the N1a components recorded at the right and left mastoid electrodes for both the /da/ and /ka/ stimuli revealed a trend towards a significant hemispheric difference, with larger responses on the left than right ($F [1,8] = 3.6$, $p=0.09$).

Table 6.2 N1 elicited by standard speech sounds (pilot and retest)

Standard	Electrode		Pilot	Retest	Paired-sample t-test	Pearson's R
Da	A1	amplitude	-1.6 (0.7)	-1.5 (0.6)	-0.2	0.07
		latency	101 (9)	105 (15)	-0.7	0.39
	A2	amplitude	-1.1 (0.7)	-1.5 (1.2)	1.5	0.8 *
		latency	99 (8)	104 (14)	-0.8	-0.63
Ka	A1	amplitude	-1.9 (0.8)	-1.7 (0.5)	-0.9	0.12
		latency	87 (8)	90 (10)	-0.6	-0.0
	A2	amplitude	-1.8 (0.7)	-2.0 (1.0)	0.7	0.68
		latency	81 (6)	86 (8)	-1.7	0.57

*** $p<0.05$ ** $p<0.01$**

Figure 6.2 ERP waveforms elicited by standard speech sounds (pilot and retest)



6.3.1.2MMN responses to speech deviants

Mismatch negativity-like responses were generated for all standard-deviant pairings (Figure 6.4). The speech ERP waveforms elicited in this experiment differ from analogous pure tone ERPs in a number of respects. Speech MMN responses in adults are of smaller magnitude, are maximal at more posterior (frontocentral rather than frontal) electrodes, and do not display inversions at temporal electrodes. One single-feature stimulus (ga), and the multiple-feature stimulus ma, generated a broad and double-peaked MMN response in both standard conditions. Double-peaked responses were not seen for any of the pure tone contrasts, nor for the ta stimulus, which generated a sharp single-peaked MMN in both standard conditions. No latency data is provided for speech MMN because of these between-stimulus contrasts. A similarity between MMN waveforms elicited by tone and speech stimuli was the absence of hemispheric asymmetry. There was no difference between the magnitude or morphology of responses at left and right electrodes in the region of maximal amplitude (C3 / C4, F3/F4, Fc3/Fc4).

Only one speech MMN standard-deviant pair demonstrated individual subject test-retest stability. This was the ta-da (voicing contrast) deviant which generated the largest response in most subjects and is therefore likely to display highest signal-to-noise ratio. There are various possible explanations for the low degree of individual stability of remaining responses. Speech MMN was of smaller amplitude than equivalent responses for pure tone contrasts, and the range of response magnitudes within the sample was relatively small. Therefore the likelihood of obtaining correlations between individual responses from the two test sessions with such a small study population is small. A potential confound reduced this likelihood still further – order of block presentation was random in both session one and session two. This confound should be removed in future studies by increasing sample size and presenting blocks in a fixed order within individual subjects at both sessions, with order counterbalanced between subjects. Despite these limitations in assessing individual stability of response magnitudes, group-level responses were stable - there were no differences in group mean response magnitudes between sessions, and the pattern of stimulus-response relationships was consistent.

Table 6.3 MMN elicited by deviant speech sounds (pilot and retest)

Standard	Deviant		Pilot	Retest	*Paired-sample t-test*	*Pearson's R*
Da	Ta	amplitude	-4.8 (2.4)	-4.6 (1.2)	*-0.3*	*-0.28*
		area	626 (202)	531 (210)	*1.7*	*0.68 **
	Ga	amplitude	-2.8 (2.3)	-2.4 (2.3)	*-0.4*	*-0.24*
		area	284 (121)	289 (149)	*-0.63*	*-0.24*
	Ma	amplitude	-5.3 (2.6)	-3.4 (2.0)	*-1.9*	*0.23*
		area	1121 (525)	1079 (328)	*0.21*	*-0.00*
Ka	Ta	amplitude	-3.1 (2.1)	-3.4 (3.1)	*0.18*	*-0.42*
		area	344 (116)	343 (243)	*0.01*	*0.21*
	Ga	amplitude	-3.4 (1.4)	-2.6 (1.8)	*-1.0*	*-0.01*
		area	291 (130)	356 (141)	*-0.9*	*-0.26*
	Ma	amplitude	-4.5 (2.7)	-3.1 (2.6)	*-1.4*	*0.26*
		area	399 (231)	380 (194)	*0.28*	*0.55*

* $p<0.05$ ** $p<0.01$ **Electrode = Cz**

Figure 6.3a ERP waveforms elicited by deviant speech sounds (pilot and retest)

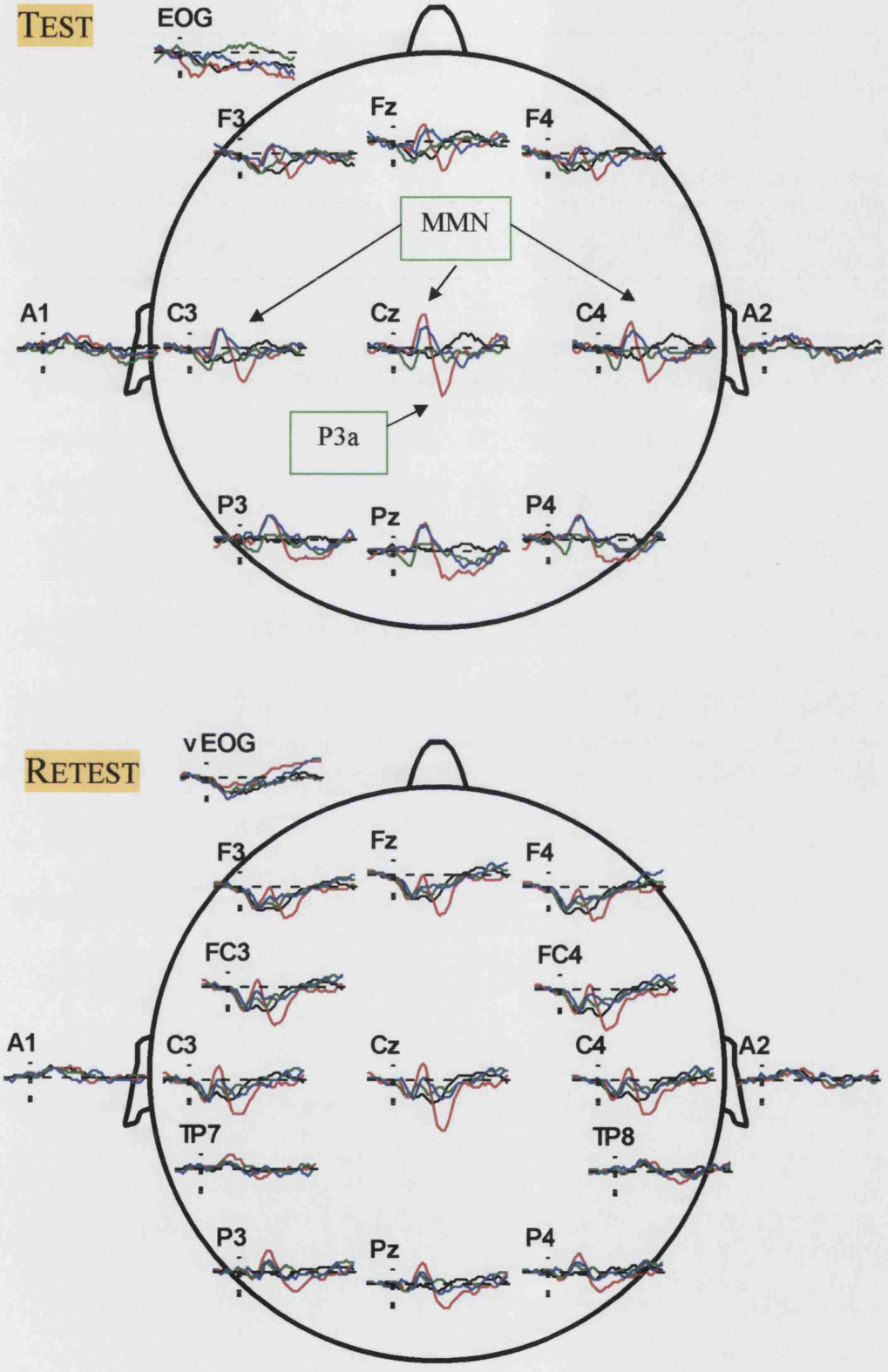
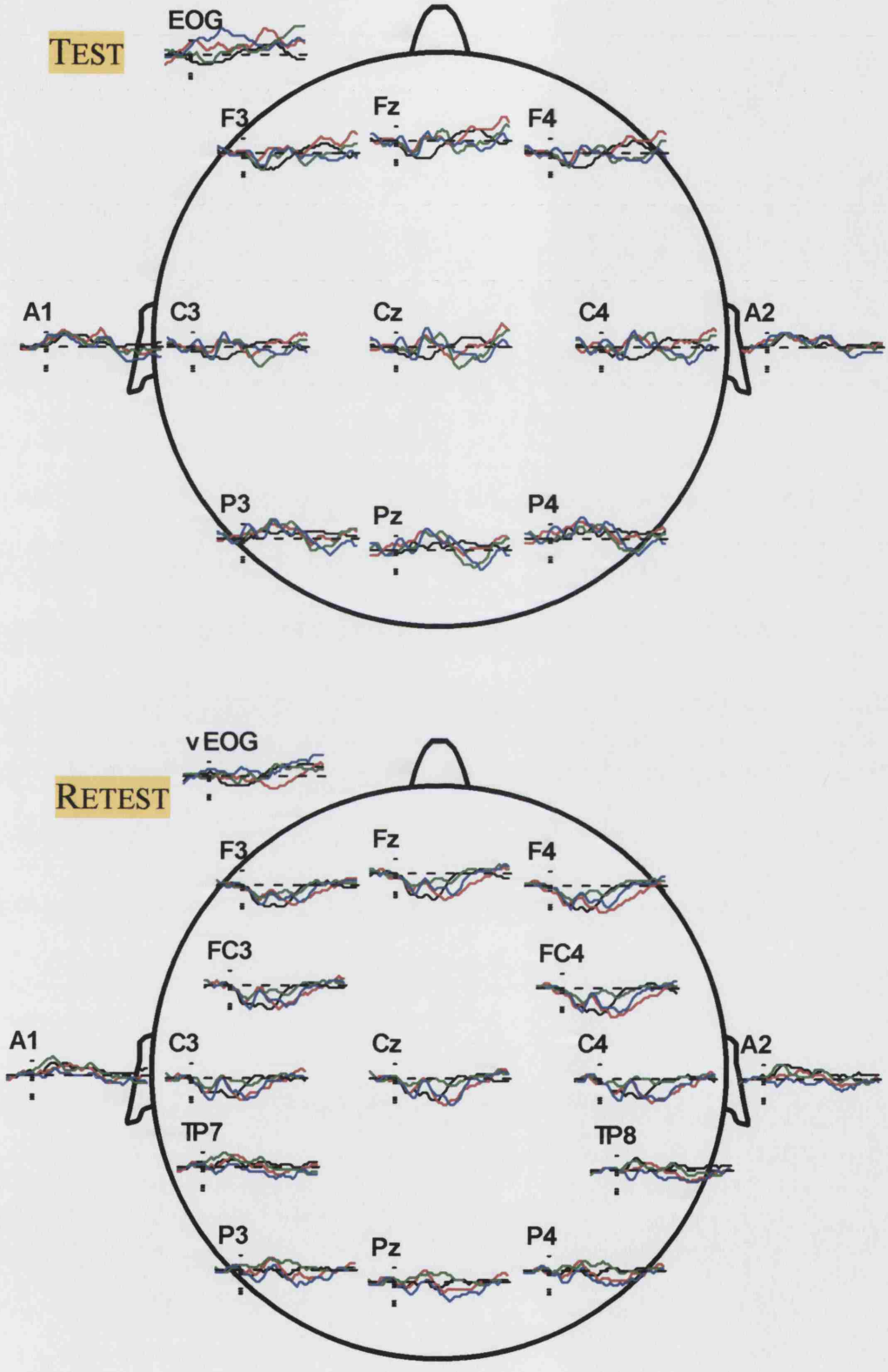


Figure 6.3b ERP waveforms elicited by deviant speech sounds (pilot and retest)



6.3.1.3P3a responses to speech deviants

Short latency (peaking between 250ms and 300ms post stimulus-onset) P3a-like responses followed the MMN, varying in magnitude in approximate proportion to the size of the MMN. P3a deflections in the subtraction waveform were quantified for standard-deviant contrasts of either voicing or multiple phonetic features. The waveform was maximal at Cz, as is the case for pure tones, suggesting that it may reflect stimulus-independent orienting. This contrasts with the topography of the MMN which differed between tone and speech sounds. However the speech P3a tended to occur earlier than for tones, particularly for the ta-da contrast.

Table 6.4 P3a elicited by deviant speech sounds (pilot and retest)

Standard	Deviant		Pilot	Retest	*Paired-sample t-test*	*Pearson's R*
Da	Ta	amplitude	7.6 (3.7)	5.5 (3.9)	*2.3 **	*0.74**
		latency	249 (50)	279 (64)	*-1.1*	*0.013*
	Ma	amplitude	5.2 (2.8)	2.8 (1.9)	*2.7 **	*0.02*
		latency	290 (31)	306 (69)	*0.6*	*0.16*
Ka	Ga	amplitude	4.3 (2.8)	3.4 (2.9)	*0.69*	*-0.03*
		latency	331 (38)	294 (39)	*2.3 **	*0.23*
	Ma	amplitude	4.2 (2.1)	4.1 (2.1)	*0.07*	*0.35*
		latency	325 (64)	278 (33)	*1.9*	*0.07*

* $p<0.05$ ** $p<0.01$ **Electrode = Cz**

6.3.1.4Contrast sensitivity of speech MMN

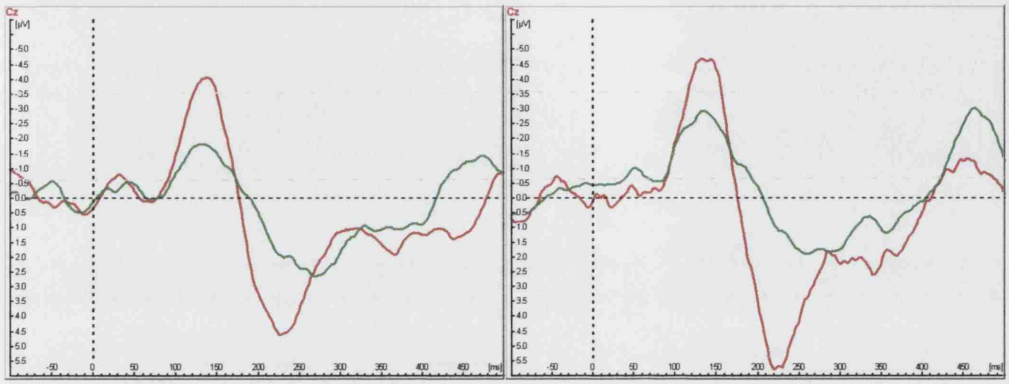
Figure 6.4 displays the subtraction (MMN) waveforms for speech sounds in both test sessions. The hypothesis that the size of the MMN response might reflect the number of phonetic dimensions in which the deviant differed from the standard was formally assessed in two GLM repeated measures analyses, entering MMN area measurements at electrode Cz from both test and retest sessions. Firstly, comparing the magnitude of MMN responses to the single deviant ta in both standard conditions to the multiple deviant ma in both standard conditions indicated a significant effect of stimulus ($F[1,8] = 26.1$, $p=0.001$). Secondly, comparing the magnitude of MMN responses to the single deviant ga in both standard conditions to the multiple deviant ma in both standard conditions also indicated a significant effect of stimulus ($F[1,8] = 54.1$, $p<0.001$). Therefore the hypothesis that speech MMN is sensitive to the magnitude of the standard-deviant contrast is supported.

The hypothesis that the size of the MMN would depend on the phonetics of the standard-deviant contrast, rather than the phonological category to which the deviant belonged, was also supported. These relationships were tested via repeated measures ANOVA for within-subject effects of session, phonetic contrast and stimulus on MMN area at Cz. There was a main effect of stimulus, with the ta deviant eliciting larger responses than the ga deviant ($F[1,8] = 23.6$, $p=0.001$). However the magnitude of the waveforms was also modulated by phonetic contrast in a consistent fashion, with standard-deviant voicing contrasts generating larger responses than standard-deviant place of articulation contrasts (effect of phonetic contrast $F[1,8]= 11.0$, $p=0.01$). There was no additional interaction between stimulus and contrast ($F[1,8] = 2.3$, $p=0.17$).

Figure 6.4 Speech MMN (pilot and retest)

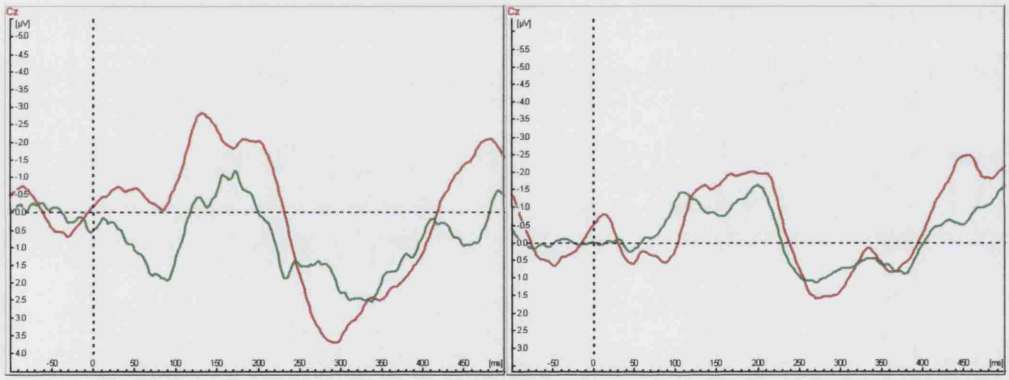
DEVIANT = TA

TEST RETEST



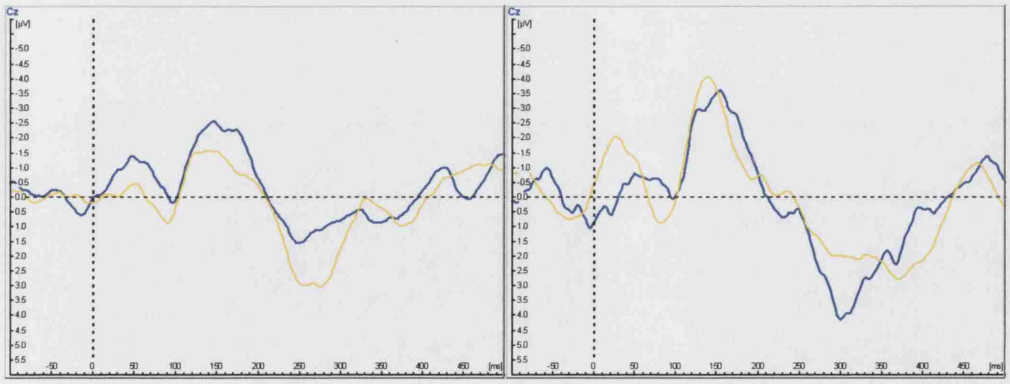
DEVIANT = GA

TEST RETEST



DEVIANT = MA

TEST RETEST



KEY

SINGLE-FEATURE CONTRASTS VOICING PLACE OF ARTICULATION

MULTI-FEATURE CONTRASTS PLACE + MANNER VOICE + PLACE + MANNER

ELECTRODE = CZ

6.3.2 Developmental trajectories

6.3.2.1N1 responses to standard speech sounds

The magnitude of N1 responses to speech sounds recorded at the mastoid electrodes declines steadily from late childhood to early adulthood, whereas latency of responses remains constant. This may reflect maturational tuning of response neurons in the auditory cortex, possibly occurring with the top-down assistance of structures in the frontal cortex. The adult data had suggested that an N1c-like second peak was only elicited by the voiceless standard (ka), but this was not the case in the younger groups, where a double-peaked response was evident for both speech standards. Repeated measures ANOVA entering the N1a and N1c components recorded at the right and left mastoid electrodes for the da standard speech stimulus (with age-group as a between subjects factor) indicated a significant hemispheric asymmetry (effect of side, $F[1,17]=4.9$, $p=0.04$), and no interaction with component. This data thus supports a left-sided dominance for the processing of speech sounds, in contrast to a lack of hemispheric asymmetry for pure tone processing.

Table 6.5 Development of speech N1

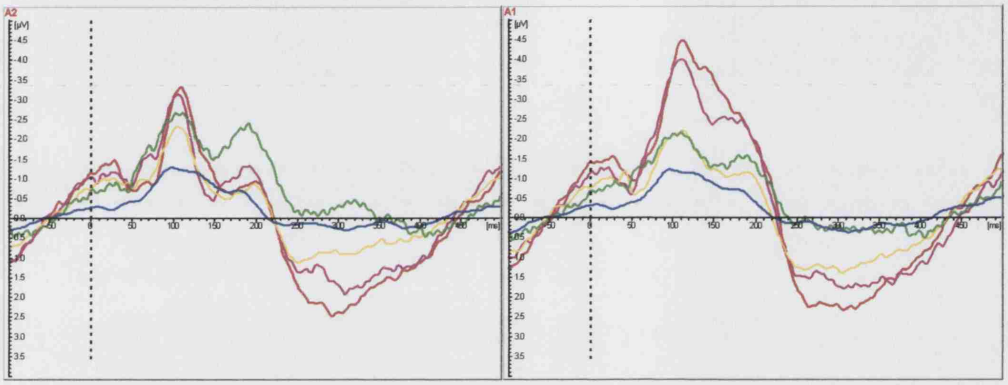
Component	Electrode		Age group 1	2	3	4	5	*Kendall's tau-b (age-group)*	*Pearson's R (age)*
N1a	A2	amplitude	-3.8 (1.1)	-3.3 (1.4)	-2.4 (1.2)	-2.6 (0.8)	-1.5 (1)	*0.39* **	*0.56* **
		latency	112 (6)	107 (5)	109 (5)	106 (9)	107 (14)	*-0.17*	*-0.14*
N1c	A2	amplitude	-2.3 (2.5)	-1.6 (1.4)	-1.2 (1.0)	-2.0 (1.7)	-1.0 (1.3)	*0.15*	*0.20*
		latency	189 (20)	182 (13)	183 (19)	174 (21)	170 (14)	*-0.31* *	*-0.38* *
N1a	A1	amplitude	-3.8 (1.1)	-3.3 (1.4)	-2.4 (1.2)	-2.6 (0.8)	-1.5 (1)	*0.39* **	*0.56* **
		latency	112 (6)	107 (5)	109 (5)	106 (9)	107 (14)	*-0.17*	*-0.14*
N1c	A1	amplitude	-4.1 (1.6)	-3.4 (1.4)	-1.3 (1.9)	-2.0 (0.6)	N/a	*0.38* *	*0.60* **
		latency	182 (17)	182 (15)	192 (13)	174 (19)	N/a	*0.00*	*0.11*

*** p<0.05 ** p<0.01, stimulus = da**

Figure 6.5 Development of speech N1

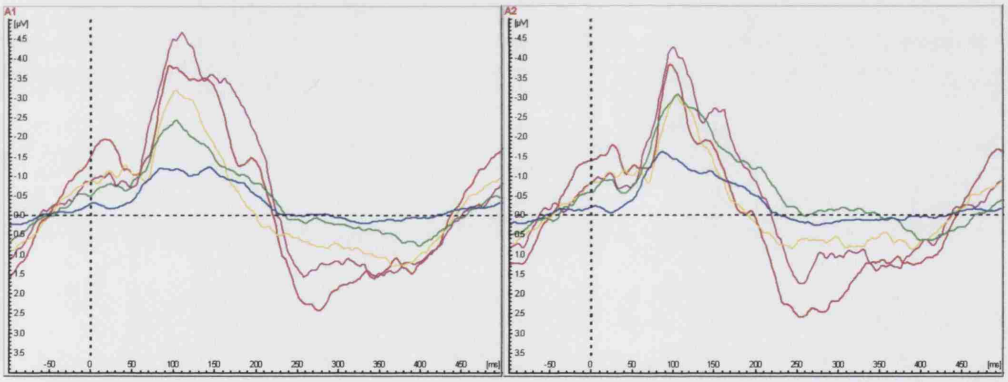
STIMULUS = DA

ELECTRODE A1 ELECTRODE A2



STIMULUS = KA

ELECTRODE A1 ELECTRODE A2



KEY GROUP: **1** ages 8 – 10 **2** ages 11-13 **3** ages 14-15 **4** ages 16-21 **5** ages 22-27

6.3.2.2MMN responses to speech deviants

For the ta deviant, which generated the most reliable MMN responses in the pilot-retest study, the voicing MMN response was found to be mature by late childhood at frontocentral electrodes. On the other hand, the frontocentral response to place of articulation contrasts decline with age, from equivalent responses to voicing contrast in childhood to significantly smaller responses by adulthood. In the younger subjects only, the voicing contrast was associated with a right-sided temporal response, showing a marked decline by mid-adolescence. MMN responses elicited by the ga deviant in the developmental sample were broadly in line with those for the ta deviant.

Table 6.6 Development of speech MMN (deviant = ta)

Contrast	Electrode		Age group					*Kendall's tau-b (age-group)*	*Pearson's Correlation (age)*
			1	2	3	4	5		
Standard = da, contrast = voicing	Cz	amplitude	-2.7 (1.6)	-7.2 (2.9)	-5.3 (1.4)	-5.0 (2.8)	-3.7 (1.3)	*0.04*	*0.05*
		area	552 (346)	630 (242)	439 (115)	443 (232)	498 (282)	*-0.11*	*-0.05*
	A2	amplitude	6.1 (4.4)	4.5 (3.6)	1.3 (2.2)	2.9 (2.3)	0.6 (0.6)	*-0.45* **	*-0.55* **
		area	720 (480)	475 (239)	320 (195)	231 (135)	139 (31)	*-0.56* **	*-0.59* **
Standard = ka, contrast = place of articulation	Cz	amplitude	-5.1 (4.1)	-4.7 (5.2)	-5.9 (2.5)	-1.7 (4.7)	-2.6 (1.6)	*0.26 (p=0.053)*	*0.3*
		area	579 (192)	561 (479)	479 (287)	515 (323)	310 (145)	*-0.29* *	*-0.32*

*** p<0.05 ** p<0.01**

Table 6.7 Development of speech MMN (deviant = ga)

Contrast	Electrode		Age group					*Kendall's tau-b (age-group)*	*Pearson's Correlation (age)*
			1	2	3	4	5		
Standard = ka, contrast = voicing	Cz	amplitude	-6.9 (2.6)	-4.3 (4.5)	-6.7 (3.0)	-3.8 (2.0)	-3.6 (2.1)	*0.29 **	*0.29*
		area	585 (95)	629 (133)	651 (223)	385 (175)	365 (133)	*-0.38 ***	*-0.44 **
	A2	amplitude	7.3 (4.3)	5.7 (4.2)	1.3 (2.7)	4.4 (2.2)	2.6 (2.3)	*-0.25*	*-0.35*
		area	591 (334)	601 (341)	381 (388)	449 (159)	243 (107)	*-0.26*	*-0.39 **
Standard = da, contrast = place of articulation	Cz	amplitude	-5.7 (3.6)	-5.9 (3.0)	-4.0 (2.5)	-2.7 (1.2)	-2.6 (2.2)	*0.35 **	*0.46 **
		area	455 (307)	531 (270)	354 (260)	286 (100)	245 (87)	*-0.28 **	*-0.39 **

*** p<0.05 ** p<0.01**

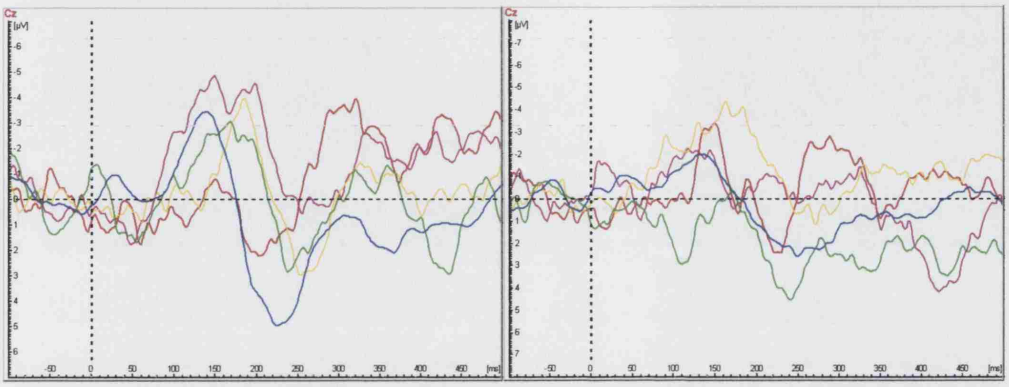
Figure 6.6 Development of speech MMN

DEVIANT = TA

VOICING MMN

PLACE OF ARTICULATION MMN

Electrode Cz

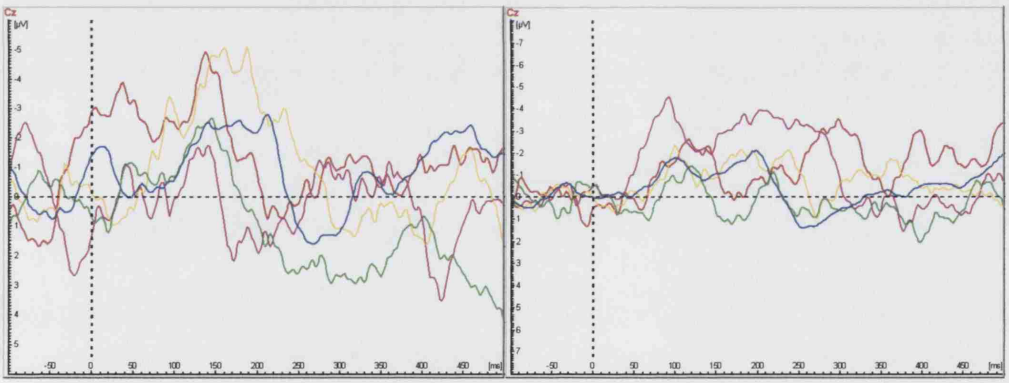


DEVIANT = GA

VOICING MMN

PLACE OF ARTICULATION MMN

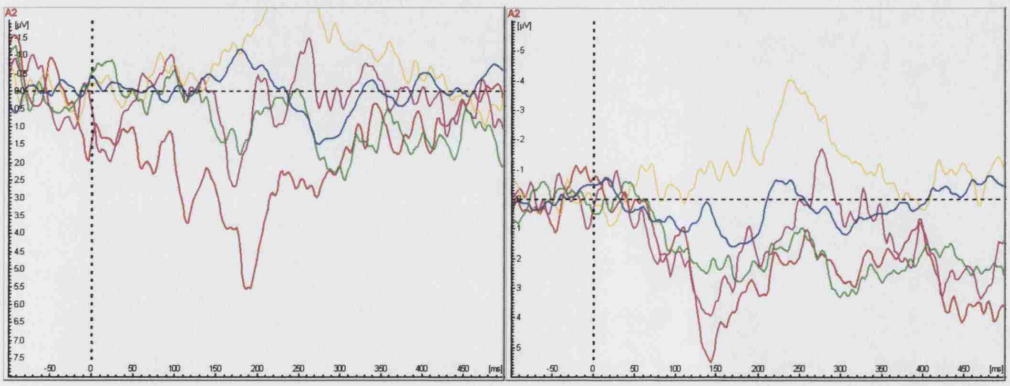
Electrode Cz



DEVIANT = TA

DEVIANT = GA

VOICING MMN AT ELECTRODE A2



KEY GROUP: 1 ages 8 – 10 2 ages 11-13 3 ages 14-15 4 ages 16-21 5 ages 22-27

6.3.2.3Development of contrast-sensitivity of speech MMN

The pattern of contrast-preference found in adulthood (larger MMN responses to voicing than to place of articulation contrasts) was replicated in the developmental sample, as indicated by a significant effect of phonetic contrast on MMN areas at Cz ($F[1,25]=5.3$, $p=0.03$) with no significant interaction between stimulus and contrast ($F[1,25] = 3.3$, $p=0.08$). Although there was no additional interaction between contrast and age-group ($F[4,25]=0.28$, $p=0.9$) examination of profile plots revealed a steady increase in the extent of the standard x deviant interaction, from absent in Group 1 to clearly present in group 5.

6.3.2.4P3a responses to speech deviants

There is no systematic development of the magnitude of P3a elicited by either tones or speech sounds. This supports the notion that this is a basic attention-related, novelty or orienting response, functionally significant in both early development and adulthood. There was a weak trend towards decreased latency of P3a response with age, perhaps reflecting a general increase in the speed of information processing, or specifically increased efficiency of processing language-relevant information. Calculating an average P3a latency (voicing and multiple speech contrasts only) for each individual confirmed that this is a weak but consistent developmental trend – Pearson's R for the correlation between average speech P3a latency was –0.29 ($p=0.1$). The possibility that this is a speech-specific developmental trend is supported by the absence of a developmental effect on the latency of pure tone P3a. Pearson's R for the correlation between average tone P3a latency (duration, frequency, duration + frequency deviants) and age was only –0.05.

Table 6.8 Development of speech P3a

Contrast	Electrode = Cz	Age group 1	2	3	4	5	*Kendall's tau-b (age-group)*	*Pearson's Correlation (age)*
Standard = ta, contrast = voicing	amplitude	5.4 (4.5)	3.3 (3.8)	3.8 (3.1)	3.7 (3.6)	5.5 (4.5)	*0.04*	*0.11*
	latency	258 (52)	279 (44)	278 (23)	255 (31)	241 (29)	*-0.24*	*-0.29*
Standard = ga, contrast = voicing	amplitude	3.3 (5.3)	5.6 (5.4)	4.6 (4.4)	5.2 (2.8)	2.5 (1.4)	*-0.09*	*-0.06*
	latency	320 (58)	283 (90)	316 (65)	314 (56)	288 (46)	*-0.06*	*-0.22*

*** p<0.05 ** p<0.01**

6.3.3 Summary of preliminary results

6.3.3.1 Stimulus-sensitivity and magnitude sensitivity of speech MMN and P3a

- Speech sounds are processed in a predominantly context-dependent manner during the early automatic, pre-attentive phase. This type of relational processing may be a general principle utilised throughout sensory systems, or may be language specific.
- Voicing contrasts between standard and deviant sounds generate larger MMN responses than do place of articulation contrasts, regardless of the specific stimuli being processed.
- In addition, the multiple-feature deviant elicited a larger MMN than either of the single-feature deviants (on average across different phonetic contrasts), indicating that context-dependent analysis of different phonetic features may take place in parallel.

6.3.3.2Test-retest reliability

- N1 responses elicited by standard speech sounds were found to be highly stable within individuals, both in terms of peak amplitudes and intra-class correlations of the complex waveform. Responses were more stable at right than at left mastoid electrodes.
- Speech MMN is a small response, especially for some stimuli and phonetic contrasts (place of articulation). Reliability of response morphology and magnitudes were high at the group level, but low at the individual level. This analysis may have been confounded by non-fixed order of block presentation. The largest response (ta-voicing) did show moderate test-retest reliability.
- P3a peak amplitudes were stable for the salient, ta-voicing contrast.

6.3.3.3Developmental trajectories

- A dissociation was observed between the maturational course of speech MMN amplitude recorded at frontocentral and temporal electrodes, the former being stable overall with increasing context-dependence, and the latter declining with age. This parallels the developmental findings for tone MMN.
- Reduced latency of the P3a response with age found in the speech experiment, but not the tone experiment, suggesting that the efficiency of language-relevant information processing occurring prior to P3a elicitation, during the phase of MMN generation, may increase through late childhood and adolescence.

6.3.4 Limitations of preliminary results

- The same limitations discussed with respect to the tone experiment regarding constitution of the adult and developmental samples (age and IQ ranges) and variation in test conditions apply here.
- This experiment utilised natural stimuli, rather than synthetically-produced speech in which acoustic parameters such as fundamental frequency and amplitude modulations can be controlled. Therefore the relationship between ERP responses and phonetic determinants such as voice-onset time and formant transitions cannot be confirmed.
- Additional issues relevant to the speech processing system that are not examined within this paradigm, which may be relevant both to schizophrenia and to developmental disorders, include vowel-processing, attentional modulation, and modulation of speech responses by linguistic context.

6.4 Case-control results

6.4.1 Behavioural data

The mean hit rates for speech discrimination in the same-different task were 90% (s.d. = 12) for control subjects and 81% (s.d.=16) in 22q11DS subjects. Performance was normally distributed in the 22q11DS group but not in controls (positively skewed). Performance was not correlated with age, IQ or average pure tone hearing threshold (whole sample Spearman's R(age)=0.28, R(IQ)=-0.09, R(pta)=-0.18). Mann-Whitney U revealed only a trend towards significant difference between groups on this task (Z=-1.8, p=0.08). Further non-parametric analysis of correct detection of voicing and place of articulation contrasts revealed no group differences for either sub-test.

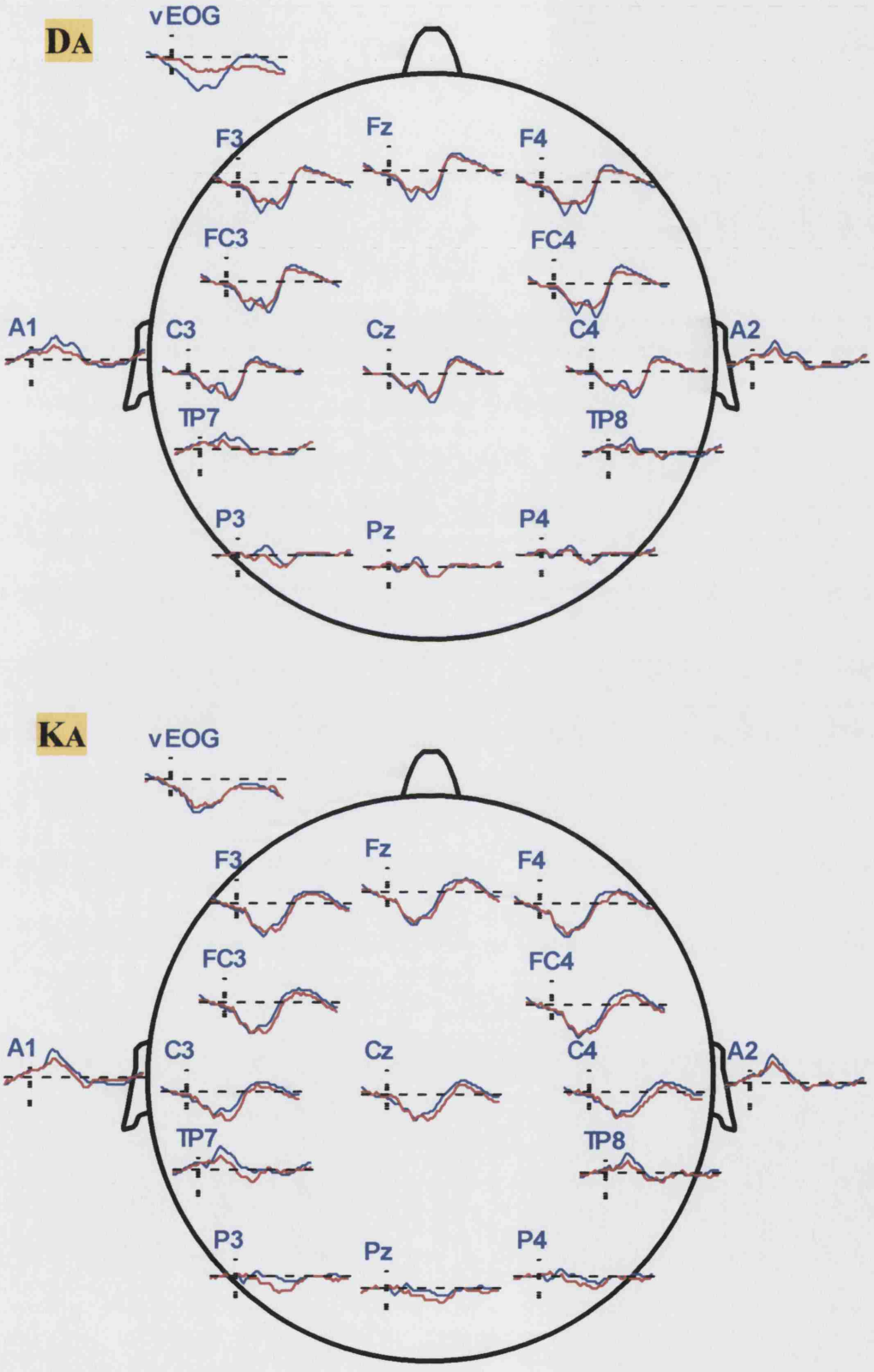
6.4.2 N1 responses to standard speech sounds

Quantification of the amplitudes and latencies of the N1a component elicited by /da/ and /ka/ stimuli at the left and right mastoid electrodes was carried out (N1c was not a distinct component of the response to the ka stimulus, Figure 6.7). Repeated measures ANOVA entering peak amplitudes for both speech standards at left and right temporal electrodes revealed a significant main effect of group on the amplitude of the speech N1a (F[1,28]=7.0, p=0.013), with no interaction between group and stimulus, nor between group and electrode. There was no similar effect of group on the latency of speech N1a (F[1,28] = 1.2, p=0.3).

Table 6.9 N1a elicited by standard speech sounds (22q11DS vs. controls)

Stimulus	Electrode		Group	
			22q11DS	**Controls**
Da	A2	amplitude	-2.0 (1.4)	-2.8 (1.1)
		latency	106 (11)	109 (6)
	A1	amplitude	-1.9 (1.0)	-3.1 (1.5)
		latency	109 (13)	104 (13)
Ka	A2	amplitude	-2.6 (1.4)	-3.6 (1.3)
		latency	111 (18)	104 (7)
	A1	amplitude	-2.2 (1.6)	-3.6 (1.4)
		latency	110 (14)	107 (10)

Figure 6.7 ERPs elicited by standard speech sounds (22q11DS vs. controls)



KEY CONTROLS 22Q11DS

6.4.3 MMN responses to deviant speech sounds

Speech ERPs are displayed in Figure 6.8, and subtraction waveforms in Figure 6.9. The group mean ERPs indicated that central mismatch-like deflections were elicited by both groups, that were more pronounced for ta and ma deviants than for ga. MMN subtraction waveforms suggested that the pattern of phonetic contrast sensitivity displayed by the two groups was not the same. In particular, responses to the ta deviant as a voicing contrast were larger in the control group than in the 22q11DS group whereas responses for ta as a place of articulation contrast were larger in 22q11DS subjects than in controls (Table 6.10).

Peak amplitudes of frontocentral MMN (at Cz) elicited by voicing and place of articulation contrasts for both ta and ga deviants were entered for all subjects into a single repeated measures analysis. This revealed no main effects of group ($F[1,30]=2.4$, $p=0.13$), contrast ($F[1,30]=1.1$, $p= 0.31$) or stimulus ($F[1,30]=1.7$, $p=0.21$). However there was a significant interaction between contrast and group ($F[1,30]=8.2$, $p=0.007$), and no additional interactions between contrast, stimulus and group. This indicates that the MMN generated in response to voicing contrasts was consistently smaller in 22q11DS subjects than in controls. This analysis remained unchanged when N1 amplitude (standard = da) was entered as a covariate (contrast x group interaction $F[1,29]=7.4$, $p=0.03$). To confirm that the group difference in the amplitude of voicing MMN was consistent across electrodes, individual amplitudes of a mean voicing waveform (combining ta-voicing and ga-voicing) were measured at additional electrodes (C3, C4, Fc3 and Fc4) and entered into repeated measures ANOVA. This confirmed the significant group difference ($F[1,28]=8.9$, $p=0.006$) with no interactions between group and hemisphere or electrode.

A mean voicing MMN amplitude (Cz) was computed for subsequent correlational analyses, combining data from ta and ga deviants, to overcome a likely effect of order of presentation. This measure was normally distributed and displayed a significant group difference ($t=-2.7$, $p=0.012$) whereas the equivalent mean place of articulation MMN measure did not differ between groups ($t=0.13$, $p=0.9$) (Figure 6.11b).

The finding that 22q11DS individuals were specifically impaired in eliciting voicing MMN was extended by analysis of responses to the multiple deviant (ma) in the two standard contexts (Table 6.10). When the standard was da (contrast = place of articulation and manner) groups did not differ in the magnitude of this response. However when the standard was ka and the contrast contained an additional voicing contrast, the control group generated a larger response than the 22q11DS group (Figure 6.10). This suggests that the 22q11DS group were insensitive to the addition of the voicing cue. Repeated measures ANOVA entering peak amplitudes of MMN for the ma deviant in both contrasts indicated a significant group x contrast interaction ($F[1,24]=5.0$, $p=0.036$) and no main effects of either group ($F[1,24]=3.2$, $p=0.10$) or contrast ($F[1,24]=2.4$, $p=0.13$). This result is driven by the failure of the 22q11DS group to elicit a larger response when the voicing cue is added to the standard-deviant contrast (Figure 6.11a).

Both groups elicited a P3a response to the multiple deviant in both standard conditions, with no group differences in the amplitude ($F[1,24]=0.26$, $p=0.61$) or latency ($F[1,24]=0.02$, $p=0.9$) of the later, attention-related component.

Table 6.10 Speech MMN (22q11DS vs. controls)

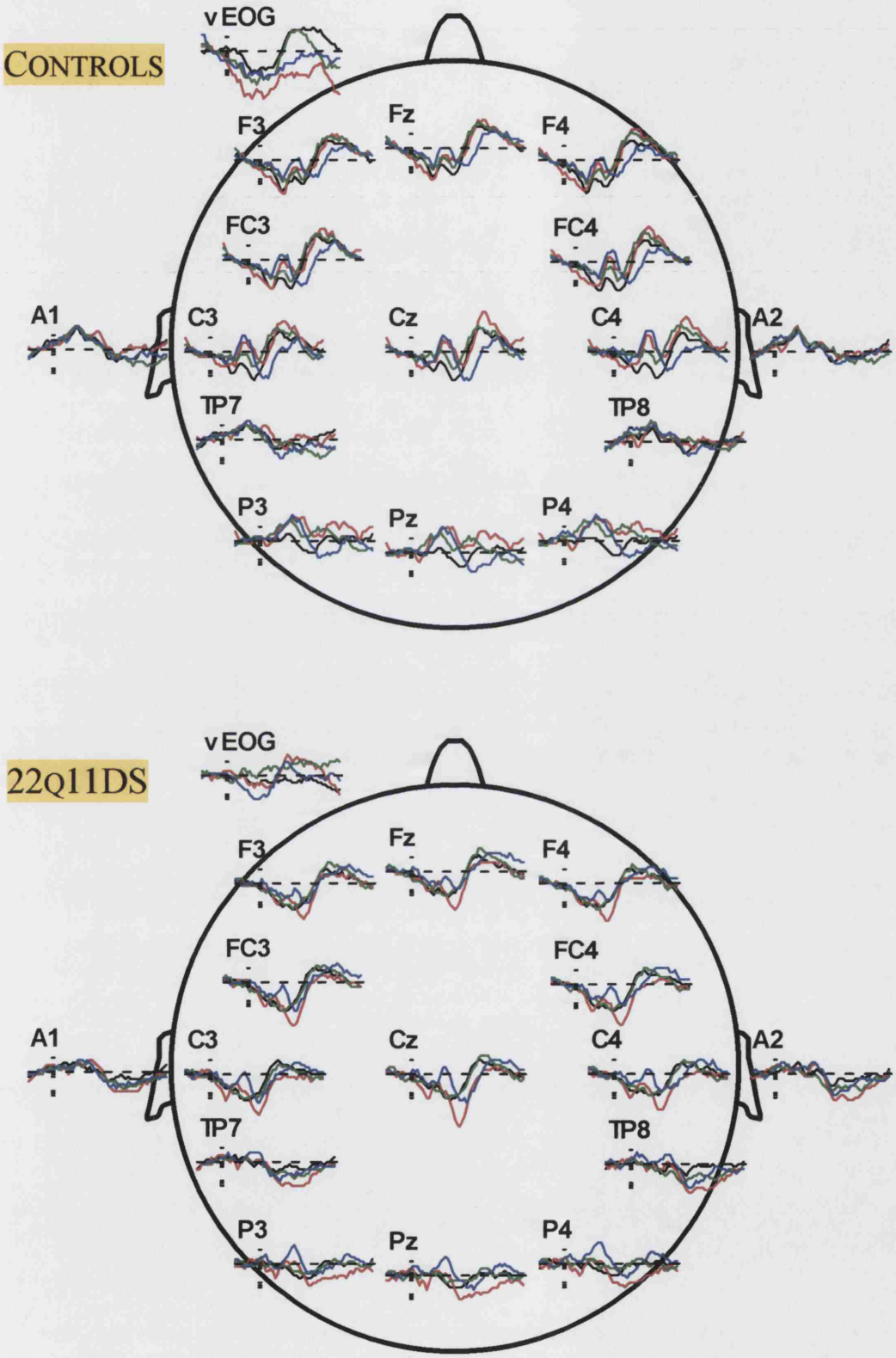
Deviant	Standard	Contrast		Group	
				22q11DS	**Controls**
ga	ka	voicing	amplitude	-3.5 (2.8)	-4.9 (3.3)
			area	425 (172)	546 (221)
	da	place of articulation	amplitude	-2.9 (2.5)	-4.0 (2.1)
			area	377 (209)	357 (210)
ta	ka	place of articulation	amplitude	-5.0 (2.6)	-3.6 (4.4)
			area	429 (174)	491 (259)
	da	voicing	amplitude	-3.4 (2.9)	-5.8 (2.6)
			area	493 (248)	503 (227)
ma	ka	voice, place and manner	amplitude	-4.0 (1.7)	-6.8 (3.0)
			area	408 (157)	598 (201)
	da	place and manner	amplitude	-4.4 (3.2)	-4.8 (2.5)
			area	412 (160)	456 (149)

Table 6.11 Speech P3a (22q11DS vs. controls)

Standard	Contrast		Group	
			22q11DS	Controls
da	place and manner	amplitude	5.1 (2.9)	5.6 (3.1)
		latency	273 (38)	270 (21)
ka	voice, place and manner	amplitude	4.6 (3.0)	4.6 (4.5)
		latency	262 (41)	266 (40)

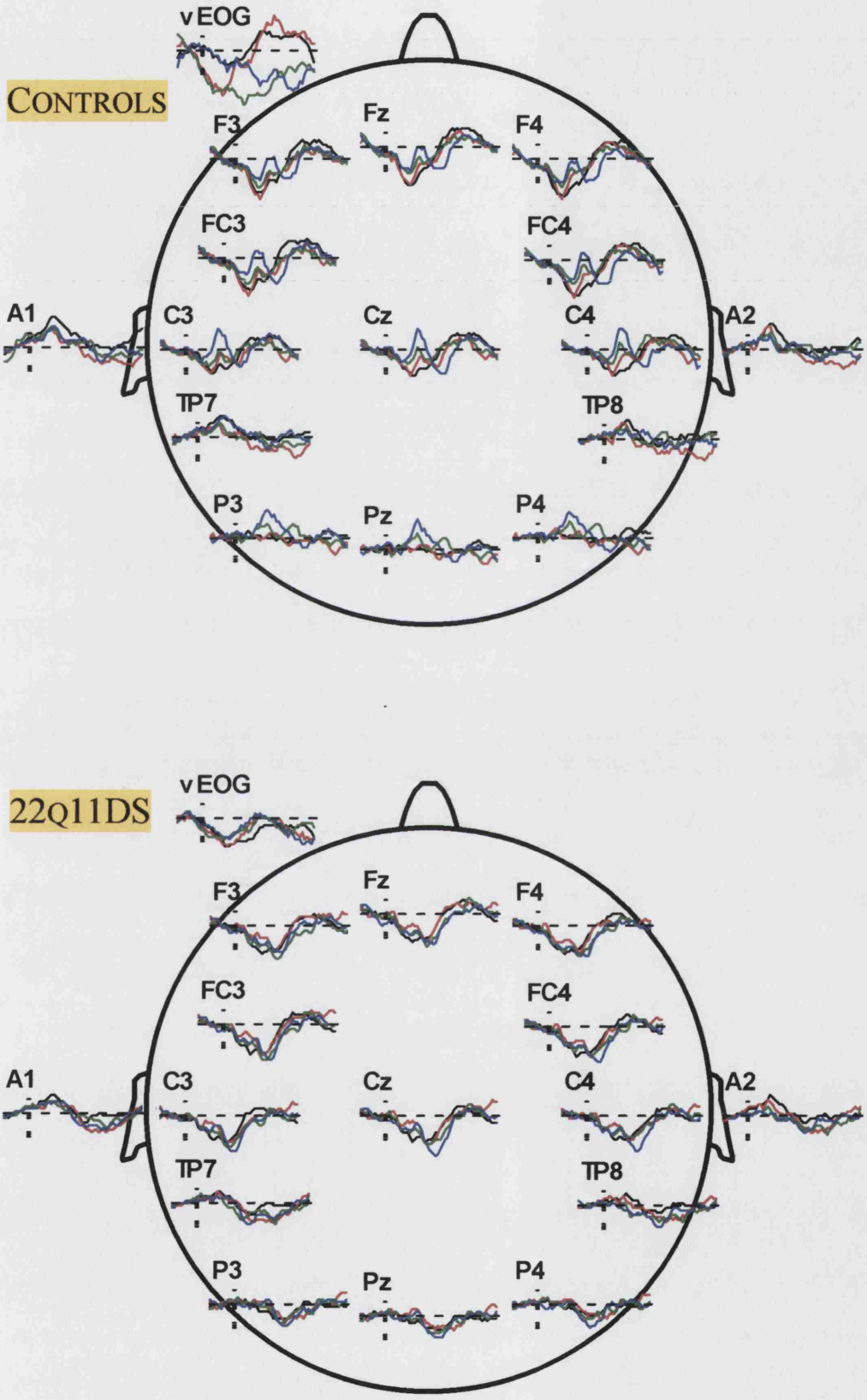
Deviant = ma, electrode = Cz

Figure 6.8a ERPs elicited by deviant speech sounds (22q11DS vs. controls)



KEY STANDARD **DA** DEVIANTS **TA** **GA** **MA**

Figure 6.8b ERPs elicited by deviant speech sounds (22q11DS vs. controls)



KEY STANDARD KA DEVIANTS TA GA MA

Figure 6.9 Speech MMN and P3a (22q11DS vs. controls)

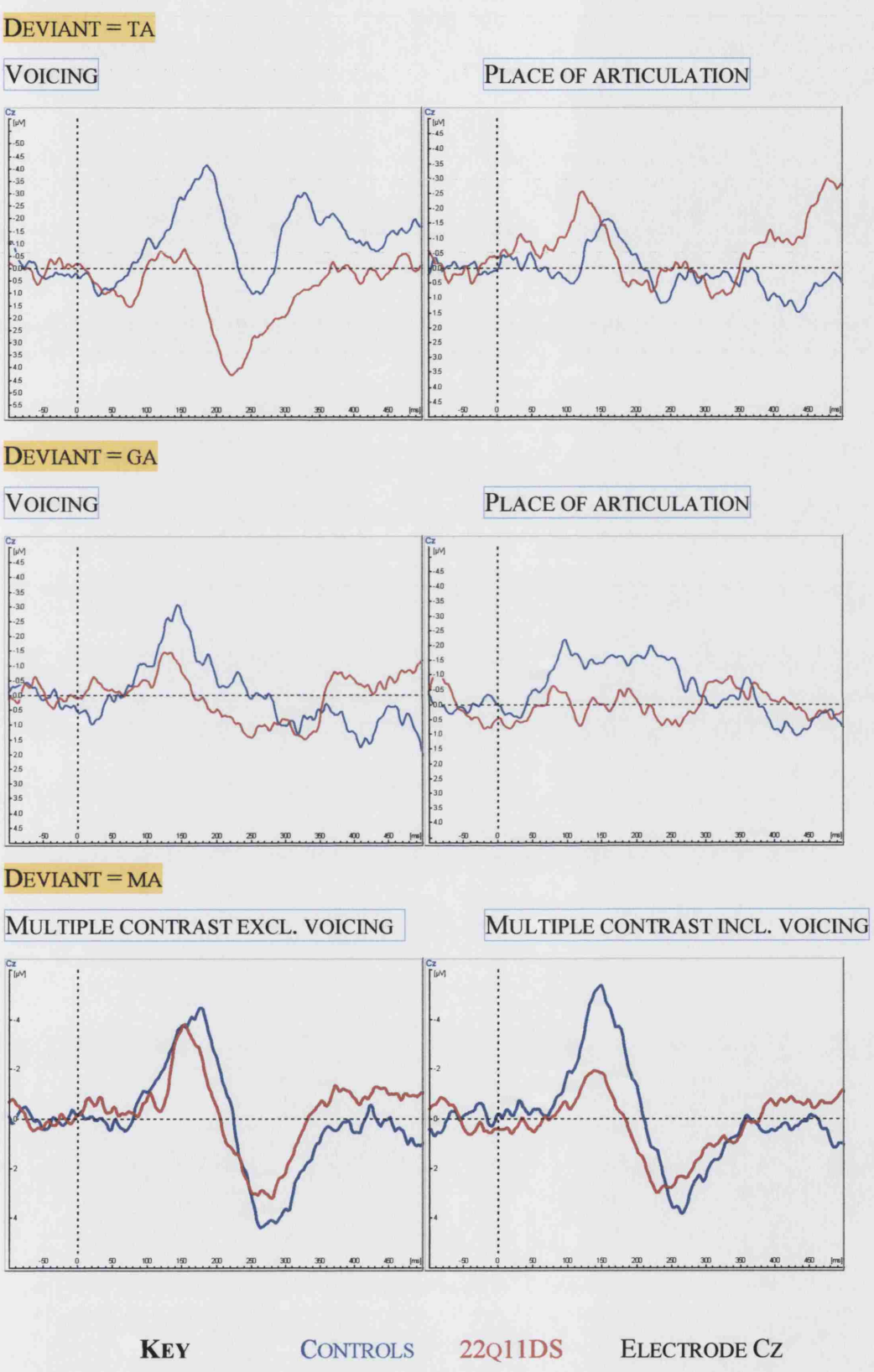


Figure 6.10a MMN sensitivity to phonetic contrasts (22q11DS vs. controls)

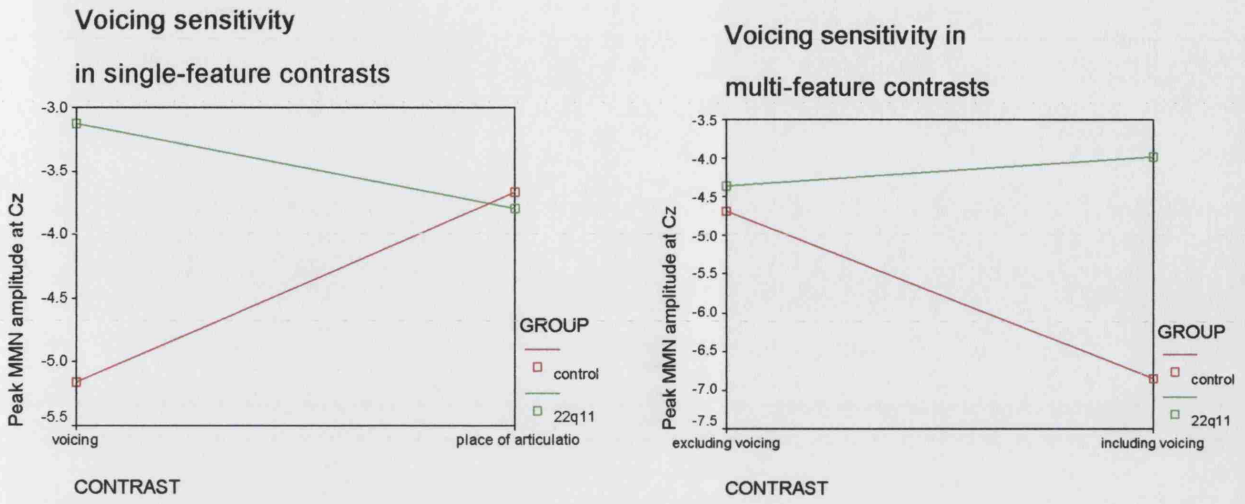
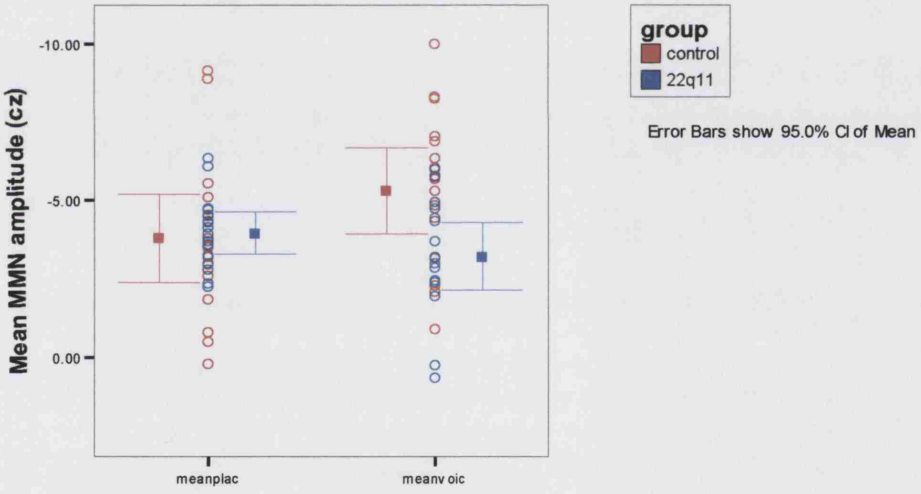


Figure 6.10b MMN sensitivity to phonetic contrasts (22q11DS vs. controls)



6.4.4 Relationships between peripheral and central auditory processing deficits in 22q11DS

Within-group non-parametric correlations were computed to assess the relationships between speech MMN deficits, input / output constraints, and central processing of basic sound features (duration and frequency).

Voicing MMN was not associated with either hearing level (Spearman's R = 0.31, p=0.3) or current speech intelligibility (Kendall's tau-b = 0.1, p=0.7) in the 22q11DS group, indicating that persistent peripheral impairments do not account for language-related neural processing abnormalities. However an impact of fluctuating hearing loss and / or articulation deficits during childhood cannot be ruled out as a component of the aetiology of this group-level difference. In future formal assessment of speech production characteristics should be conducted.

For controls, voicing MMN did not show an association with peak amplitude (at electrode Fz) of either duration (Spearman's R = 0.3) or frequency MMN (Spearman's R = 0.4). However, in 22q11DS, voicing MMN was significantly associated with duration MMN (Spearman's R= 0.6, p=0.03) and showed a trend in the same direction for frequency MMN (Spearman's R=0.5, p=0.08). This suggests that 22q11DS individuals may have difficulty in processing voicing cues in speech because of disruption of a neural circuit involved in processing many different features of change in the auditory stream. There was no evidence for similar relationships for place of articulation MMN magnitude, in either group.

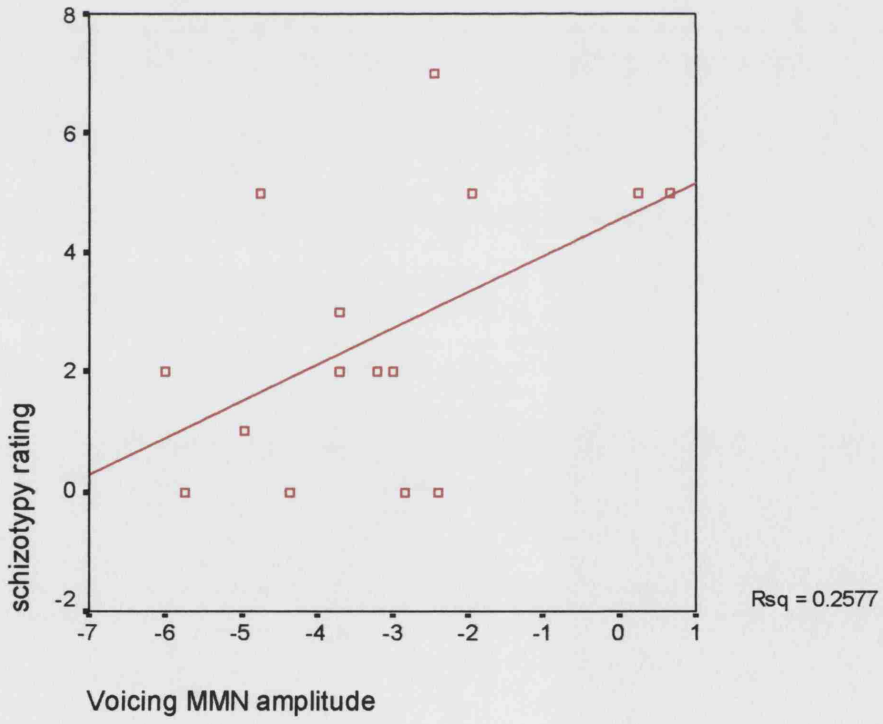
6.4.5 Relationships between MMN and cognitive abnormalities

Scatterplots were inspected to assess the potential relationships between voicing MMN / duration MMN and scores on the speech discrimination task, receptive and expressive language tasks, CCC pragmatic language index and working memory index in both 22q11DS and control subjects. No significant relationships or trends were detected for either group.

6.4.6 Relationship between voicing MMN deficits and psychopathology in 22q11DS

Spearman's non-parametric correlations were computed between the speech ERP measure showing greatest difference between 22q11DS and control groups (mean voicing MMN amplitude recorded at Cz) and the two indices of psychological function hypothetically associated with psychosis-risk (the schizotypy scale and premorbid adjustment scale). Voicing MMN was not found to be associated with IQ (Spearman's rho, controls R=0.16, p=0.6; 22q11DS R=0.23, p-0.4) therefore partial correlations are unwarranted. Analysis of the relationship between scores in the 22q11DS group revealed an association between voicing MMN and schizotypy (R=0.4, one-tailed p =0.06), but not between voicing MMN and PMA summary score (R=0.15, p=0.3). This result is consistent with the relationships detected between duration MMN and these psychological indices.

Figure 6.11 Relationship between voicing MMN and schizotypy in 22q11DS



6.5 Discussion

6.5.1 Summary

- Individuals with 22q11DS show a selective deficit in eliciting MMN in response to voicing contrasts between standard and deviant speech sounds, regardless of the absolute phonology of the stimulus or the magnitude of the standard-deviant contrast.
- 22q11DS subjects also generate smaller N1 responses to standard speech sounds. However, this deficit does not account for the group differences in speech MMN.
- Magnitude sensitivity of speech MMN (multiple contrasts eliciting larger responses than single-feature contrasts) was intact in 22q11DS, consistent with results from the tone experiment.
- Voicing MMN amplitude is correlated with duration and frequency MMN amplitude in 22q11DS subjects but not in controls. This suggests that these auditory processing abnormalities are dependent on a common neural or developmental mechanism.
- Voicing MMN deficits were found to be associated with schizotypy scores within the 22q11DS group, consistent with the association between duration MMN and schizotypy.

6.5.2 Limitations

- Test-retest reliability of speech MMN as tested in this paradigm was not strong (perhaps because mixed order of block presentation was a confounding factor). Therefore individual relationships between speech MMN and other characteristics e.g. schizotypy should be treated with caution.
- Since there was no significant group difference between groups on the speech discrimination behavioural measure, the cognitive correlates of impaired voicing MMN in 22q11DS are not known. Measures that should be included in future studies, to assess potential impact of distorted speech cue sensitivity, include nonword repetition and novel vocabulary learning tasks.
- The lack of a direct measure of phonological production skill in this experiment lessens the argument for a relationship between articulation impairments and speech MMN in 22q11DS.

- Given the large number of stimuli presented, and the confounding effect of mixed block presentation order, split-half analysis was not conducted for the speech experiment.

6.5.3 Implications

This experiment has demonstrated that auditory processing deficits in 22q11DS, detectable via the passive oddball ERP paradigm, extend from physical-acoustic deviance to phonetic deviance. There was general correspondence between the results obtained in the tones and speech experiments: as a group 22q11DS subjects elicited smaller N1 and frontocentral MMN, but not P3a, in both conditions. Within the 22q11DS group, MMN abnormalities were associated with severity of schizotypal features. Although this might appear to render this second experiment redundant, the different topographical distribution of MMN responses to speech and tone stimuli and their different stimulus-dependent properties in both groups may help to identify a common underlying mechanism, the disruption of which is associated with increased risk of psychosis. This experiment confirms that there is some degree of overlap between neural abnormalities in 22q11DS and schizophrenia (diminished frontal MMN) but that there may be divergence with respect to other ERP components and neurophysiological substrates (N1 and P3a), which may reflect either different pathways towards psychosis or trait-state interactions.

As a consequence of the experimental design employed here it has been possible to detect a specific neural insensitivity to voicing contrasts in the 22q11DS group, in both single and multi-feature standard-deviant pairings. This mirrors the report by D'Antonio et al of impaired production of voiced consonants in a sample of children with 22q11DS. However a direct relationship between productive and perceptual deficits cannot be determined without further direct assessment. If such a relationship does exist, there are at least three possible explanations for the association. Firstly, motor feedback from articulatory systems could be necessary for the organisation of perceptual systems in the brain. Secondly, impairments in detecting voicing contrasts in the acoustic stream could reduce the likelihood of imitative production of voiced sounds. Thirdly, both the production of accurately timed speech (voicing being determined in large part by voice-onset times between plosive release and vowel) and its perception could depend upon a common substrate

in the brain. This could be a shared neuroanatomical site or circuit, a common neurochemical modulator, or a shared molecular regulator of developmental plasticity, necessary for both perception and motor output. This proposal is supported by the relationship within the 22q11DS group between voicing and duration / frequency MMN deficits, although a link with speech output and language development has not been established. The lack of association between speech or tone MMN and language or working memory performance suggests that either these neurophysiological deficits do not impact directly on cognitive development, or that they are risk factors for cognitive disruption in the 22q11DS group, with outcomes being mediated or moderated by additional (environmental) factors. Further experimentation in adults and children (including children with SLI, see Chapter 7) will hopefully enhance our understanding of the mechanisms underlying speech processing and their relationship to language development.

These results also extend the observation that speech processing deficits may be a component of the neurocognitive abnormalities seen in at least some schizophrenia / psychosis patients. The previous study to utilise speech MMN as an investigative tool with regard to this question found a deficit only for speech sounds, and not for other auditory stimuli, in contrast to our findings (Kasai et al., 2003). Further investigation of the relationship between speech and basic auditory MMN deficits in schizophrenia may indicate specific stimulus-symptom associations. For example the view that auditory verbal hallucinations arise specifically as a consequence of abnormalities in the neural substrate for speech processing may be either strengthened or refuted. An alternative hypothesis suggested by our results is that speech processing abnormalities in schizophrenia arise from deficits in more basic aspects of context-dependent neural processing (detectable as diminished frontal tone MMN), likely to have widespread developmental impact on language, cognition and subjective experience.

7 Neurophysiology of auditory and speech processing – a comparison study of children with Specific Language Impairment

7.1 Introduction

7.1.1 Overview

As introduced in Chapter 6, the neurocognitive systems by which children learn language are still largely mysterious. The study of language development has been both motivated and dominated by competing theories – language modularity versus non-specialised processing capacities, nativism versus experience-dependent specialisation, bottom-up acoustic-phonetic accounts versus top-down grammatical accounts. Behavioural evidence for each account has accrued, but no argument has yet been won (nor is this likely to occur, since each proposal is likely to be true, to some extent, within its own explanatory framework). A practical purpose for these theoretical arguments is the potential application of findings to the understanding of, and provision of interventions for, individuals whose language development is delayed, deviant or disrupted relative to their acquisition of non-linguistic skills (Specific Language Impairment, SLI). In a reciprocal fashion, investigation of developmental language disorders provides one strand of evidence for models of normal language development.

Behavioural investigations have provided evidence for diverse information processing deficits, as well as language-specific deficits in phonology, morphology and syntax, in SLI (Joanisse & Seidenberg, 1998). However, the relationships between these different deficits, and between information processing abnormalities and impaired language learning, are currently very unclear. The addition of neurophysiological evidence to this arena may help to clarify the nature of information processing deficits in SLI, by identifying a common neural substrate for diverse behavioural impairments, or differentiating between behavioural impairments on the basis of distinct neural substrates. A small number of auditory ERP studies have been carried out to characterise auditory processing deficits in SLI, but results have to date been inconsistent. The design of this ERP paradigm allows for

differentiation between general auditory processing abnormalities (common to both tone and speech stimuli) and processing abnormalities specific to speech stimuli. The oddball ERP procedure allows for differentiation between sensory processes (indexed by N1 elicited by repeated standards), components of a memory-based comparison process (frontal and temporal MMN) and orienting of attention towards stimuli (P3a).

The aims of the experiment presented in this chapter were to characterise neurophysiological deficits in auditory processing for a group of children with specific language impairment (SLI), to assess the relationship between ERP and cognitive deficits in SLI. Lastly, results from the two case-control studies, 22q11DS vs. controls, and SLI vs. controls, were compared, in order to establish whether the same or different neural processing abnormalities may underlie impaired language development in the two atypical groups. These groups present a very interesting contrast for neurobiological investigation, since the 22q11DS group is defined entirely by aetiology and is behaviourally heterogeneous whilst the SLI group is aetiologically diverse but defined by behavioural similarity (albeit at a superficial level). Thus any overlap in case-control differences from the two independent studies would point towards aspects of neural processing that may be essential for normal language development. On the other hand, absence of overlap between the two groups in terms of atypical neural processing would indicate that specific pathways may be responsible for similar behavioural abnormalities in aetiologically distinct groups.

7.1.2 SLI – definition and auditory processing theory

The term Specific Language Impairment (SLI) refers to individuals who fail to develop age-appropriate receptive and / or expressive language despite being apparently normal in other respects. By definition therefore, these children have a central (neural) processing disorder, since input constraints (peripheral hearing loss) and output constraints (structural or motoric articulation impairments) are exclusionary criteria. One dominant theory is that a relatively low-level perceptual abnormality underlies vulnerable phonological representations in SLI, in line with domain-general theories for the reliance of language specialisation on basic neurocognitive capacities. Tallal and colleagues have argued that language disorders

arise from a failure to process rapid temporal changes within sound, and that although this deficit is not speech-specific, it has a marked disruptive impact on receptive and expressive language development because of the prevalence of rapid changes within speech (Tallal, 2000). Not all investigators have replicated the finding of impaired rapid auditory processing in SLI, for example it is not found in children with predominant impairments in grammar (Rosen, 1999). Other investigators have reported perceptual deficits in SLI on auditory tasks that do not involve rapid temporal change (Bishop et al., 1999) or involve visual information processing (Mills & Neville, 1997). Although auditory processing abnormalities may contribute to impairments in at least some children with SLI, the precise nature (or natures) of these abnormalities, and their relationships to language learning, have yet to be confirmed.

7.1.3 SLI – approaches to heterogeneity

Most investigators in the field of SLI accept that it is not a homogeneous disorder and that there are different routes towards developmental language dysfunction. Rapin & Allen (1988) proposed a range of SLI categories on the basis of linguistic profiles, including verbal agnosia and dyspraxia, phonological-syntactic deficit, lexical-syntactic deficit and semantic-pragmatic disorder. These categories were proposed both on the basis of contrasting profiles on test performance in language-impaired children, and on the basis of dissociations found in language performance as a consequence of neurological lesions in the adult brain. However, finding children that conform neatly to the definition of one category and that stay in the same category over time has proven difficult (Conti-Ramsden & Botting, 1999). This suggests that a complex, dynamic and interactive system mediates language development in the brain, such that overlapping linguistic deficits arise as a consequence of different underlying neurocognitive disruptions, and that the profile of linguistic deficits resulting from underlying disruption changes over developmental time (Bishop, 1997).

An alternative way to classify SLI cases would be on the basis of contrasting cognitive disruptions. Neuropsychological data and clinical observation consistently show that the functional deficits seen in children with SLI are rarely, if ever, entirely specific to language. For example, children with SLI can also show deficits in

attention, short and long-term memory skills, numeracy, motor skill and visual perception (reviewed in Bishop 1997). Some of these deficits could arise as consequences of poor language attainment and impaired engagement with education (for example mathematical ability) or as a direct cognitive consequence of impaired language (for example verbal ability might be necessary for aspects of episodic memory). On the other hand co-morbidity of deficits could indicate common neurocognitive pathways towards different functional problems, for example adequate auditory processing could be necessary for both language learning and working memory function.

Lastly, non-linguistic deficits in SLI could indicate causal pathways, for example adequate working memory capacity may be a precursor for vocabulary acquisition (Gathercole & Baddeley, 1990). Rather than attempting to find discrete subtypes of children with SLI, either on the basis of language profile or neuropsychological deficits, it may be more informative, with regard to processes and mechanisms, to look for relationships between neural, cognitive and behavioural characteristics within a heterogeneous sample. Our hypothesis is this: a child's ability to learn language may be constrained by disruption of any one of a number of processes, and identification of these processes may be achieved by seeking convergence between neural and cognitive variables, within a population showing a broadly similar profile of language impairment.

7.1.4 SLI – neurophysiological evidence for an auditory processing component

As mentioned above, a small number of ERP studies have been conducted to determine the presence and nature of basic auditory processing abnormalities in SLI. Comparison between studies is hampered by differences between study populations (and in some cases inadequate description of population characteristics, for example with respect to non-verbal intelligence), experimental design and EEG recording and processing methodology. Korpilahti et al (1994) reported reduced amplitude of mismatch negativity in two groups of SLI children, one with severe impairments and one more mild. They suggest that impairments are more marked for pitch change than for duration change, since a group difference for the latter deviant-type was limited to a very large standard-deviant difference. This finding was partially

replicated by Holopainen and colleagues (1997) – children with receptive language disorder differed from normal controls in terms of frequency MMN magnitude, but similar deficits were seen in children with moderate mental retardation (Holopainen, Korpilahti, Juottonen, Lang, & Sillanpaa, 1998). Whilst the authors of this study conclude that MMN deficits characterise language impairments, with or without additional deficits in non-verbal cognitive ability, the similarity between these two groups questions the specificity of MMN deficits as indicative of specific vulnerability of language systems.

Lastly, Uwer et al (2002) found that children with SLI did not demonstrate frequency or duration MMN deficits, but did elicit smaller responses than controls for speech deviants. This data was obtained using a phonological MMN experiment similar to the one employed in the current investigation, except that only place of articulation contrasts were presented, which we have found to elicit very small MMN in both adults and children. The speech ERP deficits reported by Uwer et al refer to broad waveforms in a very late latency window (200-700ms), arguably much too late to be termed MMN. The relationship between ERP abnormalities and cognitive processing abnormalities in SLI are also unclear – Uwer et al found no correlation within the SLI group between MMN measures (both speech and non-speech) and performance on a same-different discrimination task. Other studies did not collect behavioural data thus the potential relationship between MMN deficits and perceptual processing has not been addressed. An additional limitation of previous studies is that EEG recordings have been referred to linked mastoid electrodes, precluding analysis of temporal components of waveforms elicited by both standard and deviant stimuli. Given the evidence from this and other investigations that temporal N1 and MMN responses have different stimulus-dependent and developmental characteristics from their frontocentral counterparts, important SLI-associated differences may not yet have been assessed.

The question of heterogeneity of neurocognitive disruption and associated ERP anomalies within SLI populations has been addressed in only one published study (to our knowledge). Neville (1993) reported that N1 abnormalities were present only in SLI subjects who demonstrated poor performance on Tallal's Auditory Repetition Test. However Neville et al did not measure MMN in their study, nor did they report relationships between N1 and other cognitive tasks, so the specificity of any

relationship between N1 and rapid perceptual processing cannot be claimed. In a study of children (without SLI) aged 7-9 years (Ceponiene et al 1999), poor performers on a pseudoword repetition task elicited smaller speech MMN, but did not differ in the magnitude of pure tone pitch MMN. This suggests that speech MMN reflects activity in speech-specific circuits directly involved in phonological short term memory, or impacting on the establishment of phonological representations utilised when processing speech within working memory. Thus speech MMN could be a neural marker for the deficit in phonological loop capacity proposed by Gathercole and Baddeley (1990) to be a limiting factor for vocabulary development in SLI. The hypothesis that working memory and correlated MMN abnormalities could define a subgroup of children with SLI has not yet been addressed.

7.1.5 Aims of this study

The aims of this study were threefold. Firstly, the presence and nature of deficits in auditory and speech processing that can be identified in a group of children with SLI using ERP methodology needs to be clarified, given contradictions within the existing literature. It is possible that the paradigm designed for the 22q11DS study may assist in this endeavour, since it enables comparisons between speech and non-speech responses, at multiple topographical sites. Secondly, relationships were examined between ERP abnormalities (N1 and MMN) and language-relevant cognitive skills (auditory discrimination and working memory) in children with SLI. Such an analysis has not been conducted before, because this range of ERP and cognitive variables has not previously been measured within one study. Lastly, results from the two independent case-control studies (22q11DS, SLI) were compared, to establish what similarities and differences in ERP abnormalities exist, in individuals sharing some developmental characteristics (language disorder) but contrasting in both aetiology and other associated impairments. By comparing different groups, selected either on the basis of common behavioural characteristics (SLI) or on the basis of common aetiology (22q11DS), the specificity of ERP-cognitive relationships for different clinical populations can be determined.

7.2 Results

7.2.1 ERP data – tone experiment

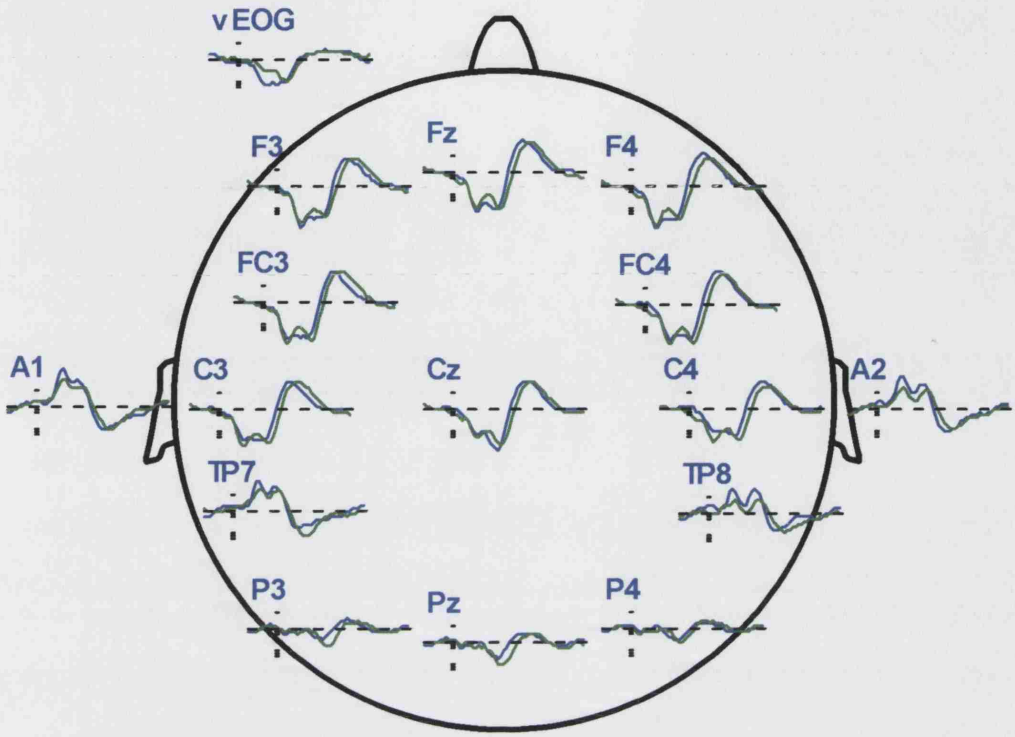
7.2.1.1 N1 responses to standard tone

A double-peaked response (N1a and N1c) to the pure tone standard stimulus was seen in SLI and control subjects, at both left and right temporal electrodes (maximal at A1 and A2). GLM repeated measures ANOVA was conducted, entering N1a and N1c amplitudes from both left and right mastoid electrodes, to identify any consistent group differences in obligatory ERP responses associated with the repeated presentation of the tone standard, and any altered asymmetry in the SLI group. This revealed a trend towards a main effect of group ($F[1,16]=3.4$, $p=0.09$), with no evidence for a group x component or group x electrode interaction. A similar analysis for the latency of N1 peaks revealed no main effect of group ($F[1,16]=3.0$, $p=0.1$) and no interactions between group and either component or electrode.

Table 7.1 N1 elicited by standard tone (SLI vs. controls)

Component	Electrode		Group	
			Controls	**SLI**
N1a	A2	amplitude	-3.9 (1.2)	-2.9 (1.5)
		latency	102 (9)	107 (13)
N1c	A2	amplitude	-3.6 (2.2)	-2.8 (1.4)
		latency	171 (17)	193 (11)
N1a	A1	amplitude	-4.3 (1.6)	-3.3 (1.5)
		latency	103 (9)	108 (13)
N1c	A1	amplitude	-3.0 (2.1)	-2.0 (1.5)
		latency	178 (16)	181 (13)

Figure 7.1 ERP waveforms elicited by standard tone (SLI vs controls)



KEY CONTROLS SLI

7.2.1.2 MMN responses to deviant tones

Grand average ERP and subtraction waveforms for the duration deviant indicated pronounced frontal and temporal MMN components in both control and SLI groups. There was no observed group difference in amplitude (t=0.39, p=0.0.7) or latency (t=-0.32, p=0.75) of the frontal duration MMN. However the MMN peak at temporal electrodes appeared to be of longer latency in the SLI group. In contrast to the duration deviant, a clear frontal MMN was not elicited in response to frequency change in either group. The 50Hz difference between the standard and the deviant may have been too small for children of this age to process in a reliable fashion. Therefore no conclusions can be drawn as to the relative accuracy of pitch processing in SLI children as reflected by this ERP. However, a temporal mismatch deflection in response to frequency deviation was visible in both groups, which appeared to show a latency difference between groups similar in magnitude to that observed for the duration deviant.

Repeated measures ANOVA entering peak amplitudes from six temporal electrodes grouped into right (A2, P8 and Tp8) and left (A1, P7 and Tp7) hemispheres for both duration and frequency deviants revealed no difference between groups ($F[1,22]=0.08$, $p=0.79$). A similar analysis entering peak latencies at the same six electrode sites revealed a significant main effect of group ($F[1,22]=11.2$, $p=0.003$) and no interactions between group and stimulus or between group and hemisphere.

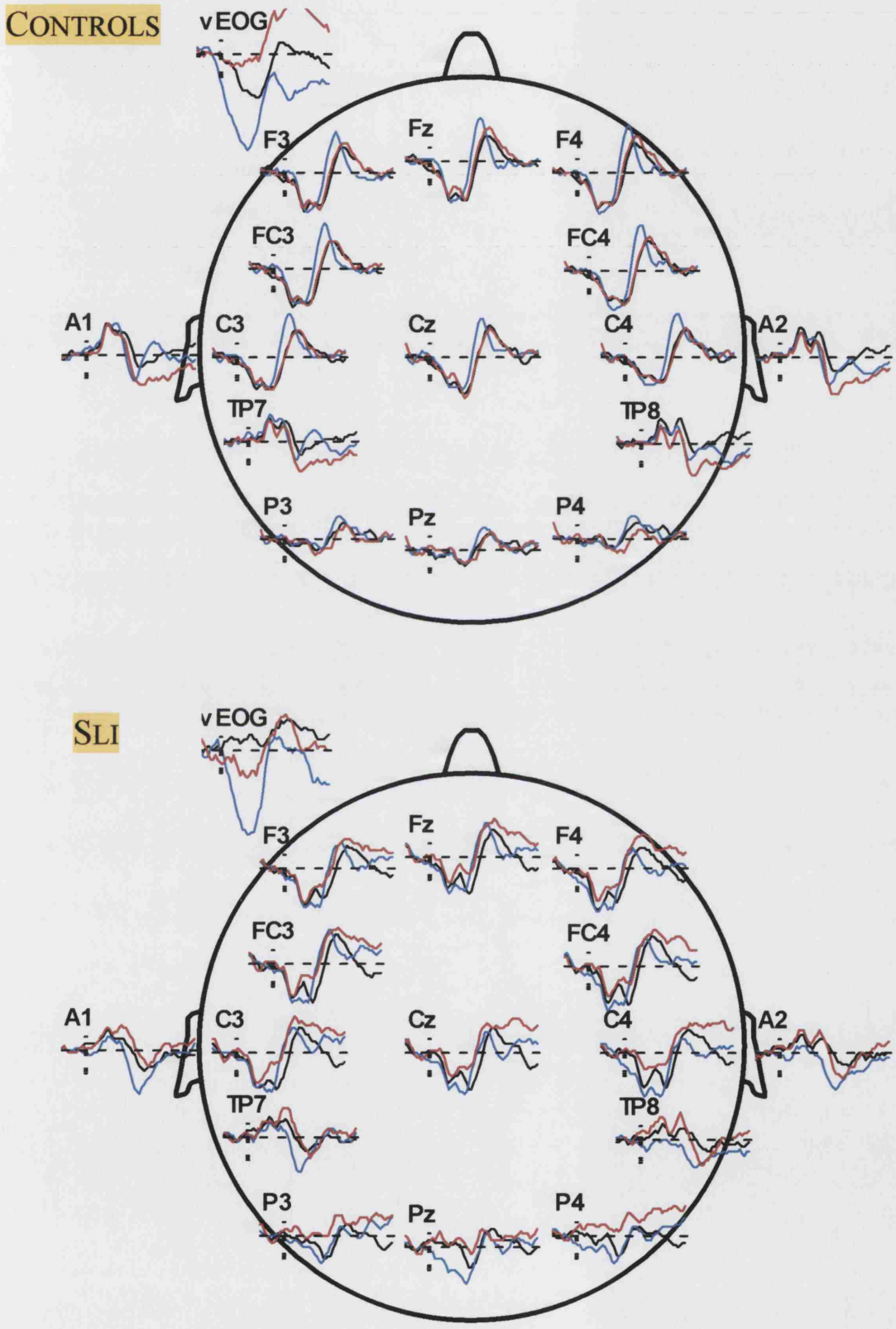
A small P3a-like positive deflection followed MMN for the duration deviant, maximal at Cz. This did not differ between groups in either amplitude (controls = 4.8μV s.d. 3.7; SLI = 5.6μV s.d. 4.6; $t=0.5$, $p=0.6$) or peak latency (controls = 326ms s.d. 40; SLI = 331ms s.d. 39; $t=0.6$, $p=0.6$).

Table 7.2 MMN elicited by tone deviants (SLI vs. controls)

Deviant	Electrode		Group	
			Controls	SLI
Duration	Fz	amplitude	-4.8 (2.6)	-5.3 (3.9)
		latency	205 (22)	209 (43)
	A2	amplitude	5.1 (4.1)	6.9 (3.2)
		latency	213 (23)	235 (22)
Frequency	Fz[a]	amplitude	-3.3 (1.7)	-5.2 (3.1)
		latency	172 (60)	188 (57)
	A2	amplitude	4.4 (3.2)	4.0 (4.8)
		latency	257 (41)	288 (47)

[a] waveforms did not show a consistent MMN-like morphology

Figure 7.2 ERP waveforms elicited by deviant tones (SLI vs. controls)

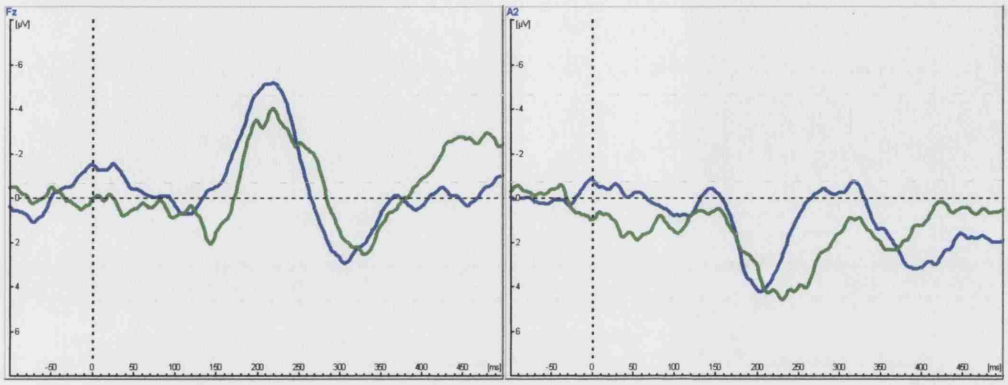


KEY STANDARD DURATION DEVIANT FREQUENCY DEVIANT

Figure 7.3 Tone MMN (SLI vs. controls)

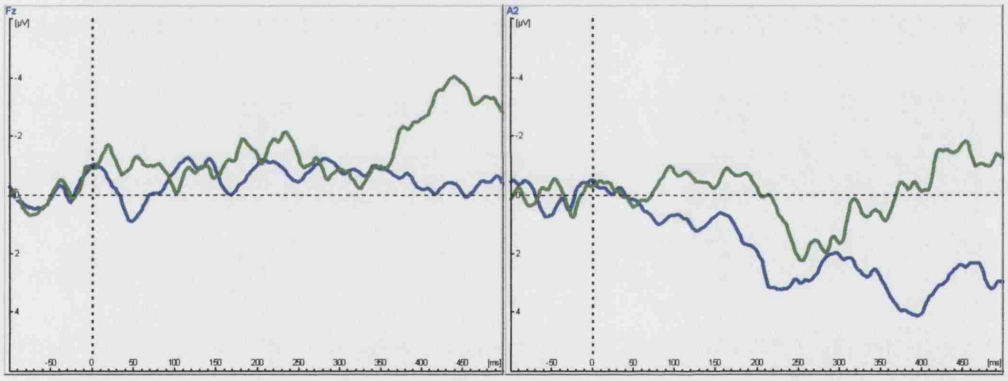
DEVIANT = DURATION

ELECTRODE FZ ELECTRODE A2



DEVIANT=FREQUENCY

ELECTRODE FZ ELECTRODE A2



KEY CONTROLS SLI

7.2.2 ERP data – speech experiment

7.2.2.1N1 responses to standard speech sounds

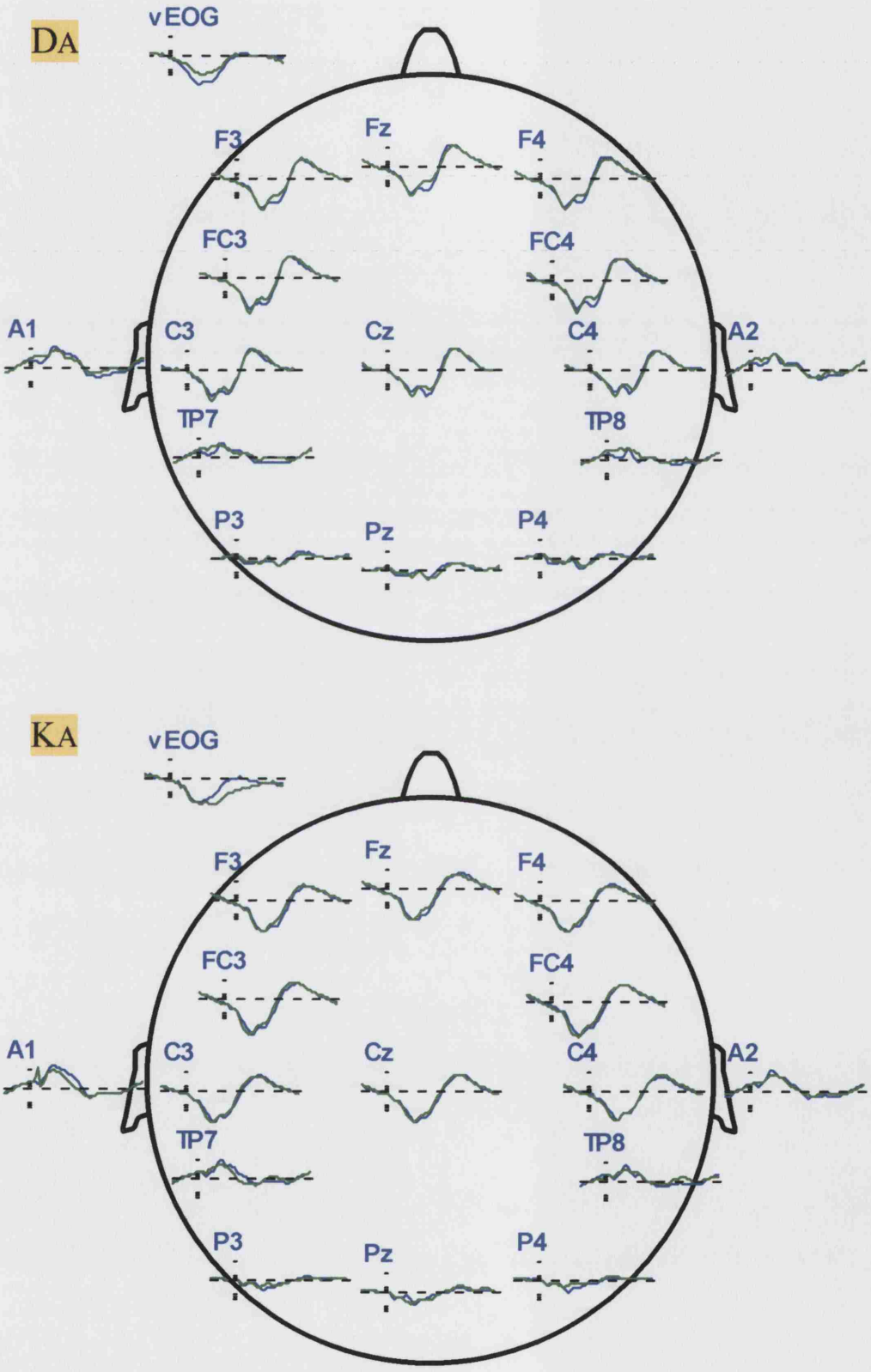
GLM repeated measures ANOVA was conducted to assess amplitude and latency changes in the N1 waveform (N1a and N1c components) across left and right temporal electrodes (for standard stimulus da). A trend towards a main effect of group was found for the amplitude of speech N1 ($F[1,15] = 3.4$, $p=0.09$), with no interactions between group and electrode or group and N1 sub-component.

Table 7.3 N1 elicited by standard speech sound (SLI vs. controls)

Component[a]	Electrode		Group	
			Controls	**SLI**
N1a	A2	amplitude	-3.6 (1.2)	-3.5 (2.6)
		latency	110 (6)	107 (11)
N1c	A2	amplitude	-1.9 (2.0)	-1.2 (2.2)
		latency	186 (16)	184 (23)
N1a	A1	amplitude	-4.4 (2.2)	-3.9 (2.3)
		latency	110 (8)	114 (15)
N1c	A1	amplitude	-3.7 (1.5)	-1.8 (2.2)
		latency	182 (15)	201 (14)

[a]**stimulus = da**

Figure 7.4 ERP waveforms elicited by speech standards (SLI vs. controls)



7.2.2.2 MMN responses to deviant speech sounds

Observation of grand mean ERP and difference waveforms elicited by speech deviants (Figures 7.5 and 7.6) indicated that ta and ga were associated with a small frontocentral MMN when presented as voicing contrasts in both experimental groups. A similar frontocentral peak was visible for ta as a place of articulation contrast but much less distinctly for ga in this phonetic context. GLM repeated measures ANOVA entering peak amplitudes at Cz for both stimuli (ta and ga) and both contrast-types (voicing and place of articulation) revealed no main effect of group ($F[1,22]=0.3$, $p=0.6$) and no contrast x group ($F[1,22] = 0.01$, $p=0.9$), or stimulus x group ($F[1,22] = 3.1$, $p=0.1$) interactions.

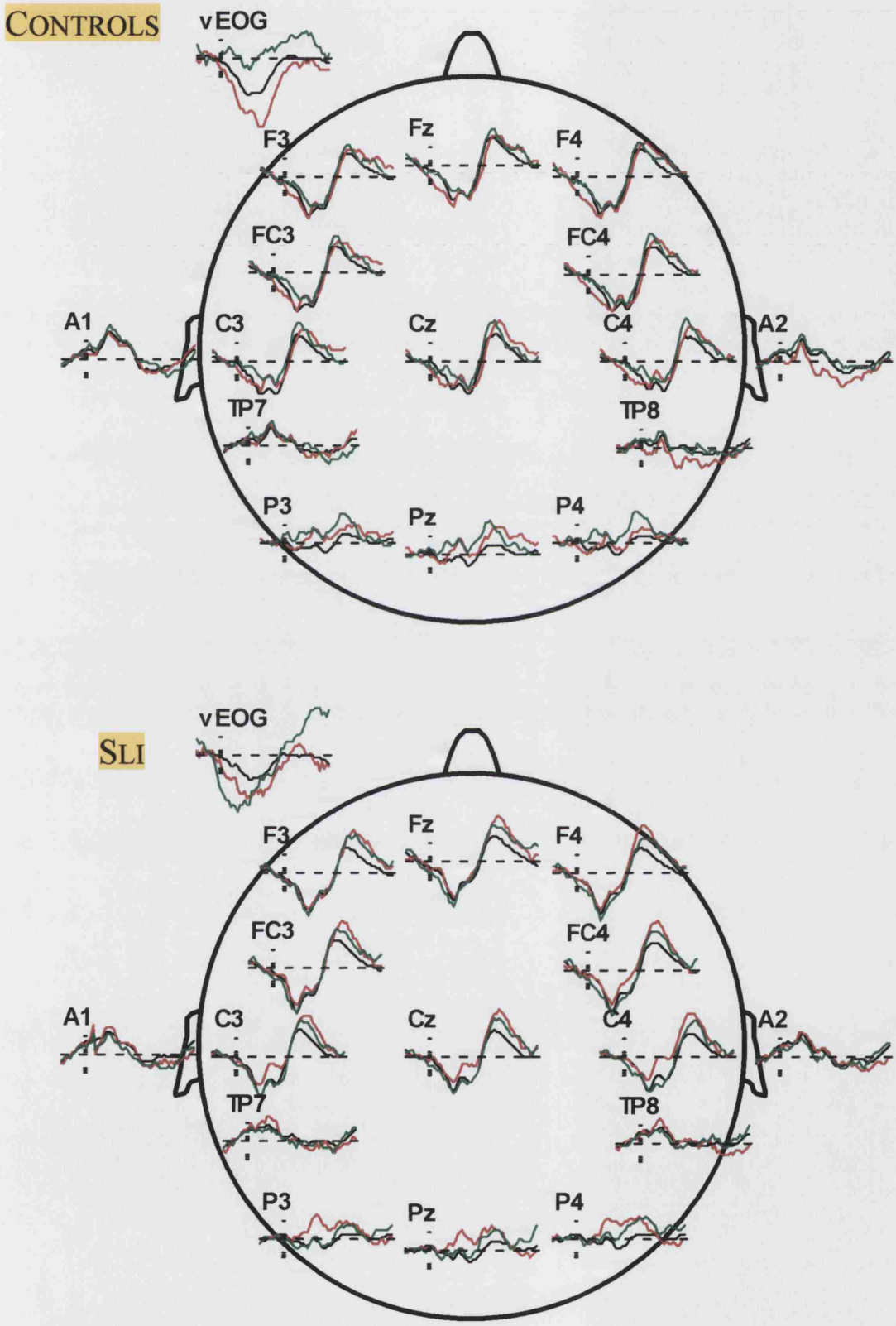
In contrast to the lack of between-groups or within-subject differences for frontocentral speech MMN, responses at temporal electrodes revealed a striking difference between groups. A positive-going peak was elicited by control subjects in right-sided temporal electrodes for both stimuli in the context of a voicing contrast, but not in the context of place of articulation contrast. This peak was absent in the grand mean waveforms for the SLI group. GLM repeated measures entering peak MMN amplitudes at electrode A2 for ta and ga indicated a significant between-groups effect ($F[1,22]=6.0$, $p=0.02$), and no stimulus x group interaction ($F[1,22]=0.3$, $p=0.6$).

To control for possible skewing of peak amplitude measurements by drift in the somewhat noisy signal in both groups, mean amplitudes were computed in the time-window 100-200ms post stimulus-onset. Repeated measures ANOVA for the mean amplitude at electrodes A2, Tp8 and P8, for both ta and ga as voicing deviants, confirmed this between-groups effect ($F[1,22]=4.2$, $p=0.05$). Entering N1a amplitude for the da standard as a covariate into this analysis did not change the strength of this effect ($F[1,21] = 4.0$, $p=0.06$). Entering age into this analysis, since the temporal voicing MMN was seen to decline in magnitude with age in the developmental data, did not have any effect ($F[1,21]=4.7$, $p=0.042$). The average of the mean amplitude measurements at electrodes A2, Tp8 and P8 was taken as a single measure of temporal voicing MMN magnitude for further analyses.

Table 7.4 Speech MMN (SLI vs. controls)

Deviant	Standard	Contrast	Electrode		Group	
					Controls	SLI
ta	da	Voicing	Cz	amplitude	-4.9 (3.2)	-4.6 (4.1)
			Tp8, P8, A2	mean amp	1.2 (4.8)	-1.2 (2.7)
	ka	Place of articulation	Cz	amplitude	-4.9 (4.5)	-6.5 (4.3)
ga	ka	Voicing	Cz	amplitude	-5.6 (3.8)	-5.1 (3.1)
			Tp8, P8, A2	mean amp	2.5 (3.5)	0.8 (3.1)
	da	Place of articulation	Cz	amplitude	-5.8 (3.2)	-3.1 (4.5)

Figure 7.5a ERPs elicited by speech stimuli (SLI vs. controls)



KEY STANDARD **DA** DEVIANTS **TA** **GA**

Figure 7.5b ERPs elicited by speech deviants (SLI vs. controls)

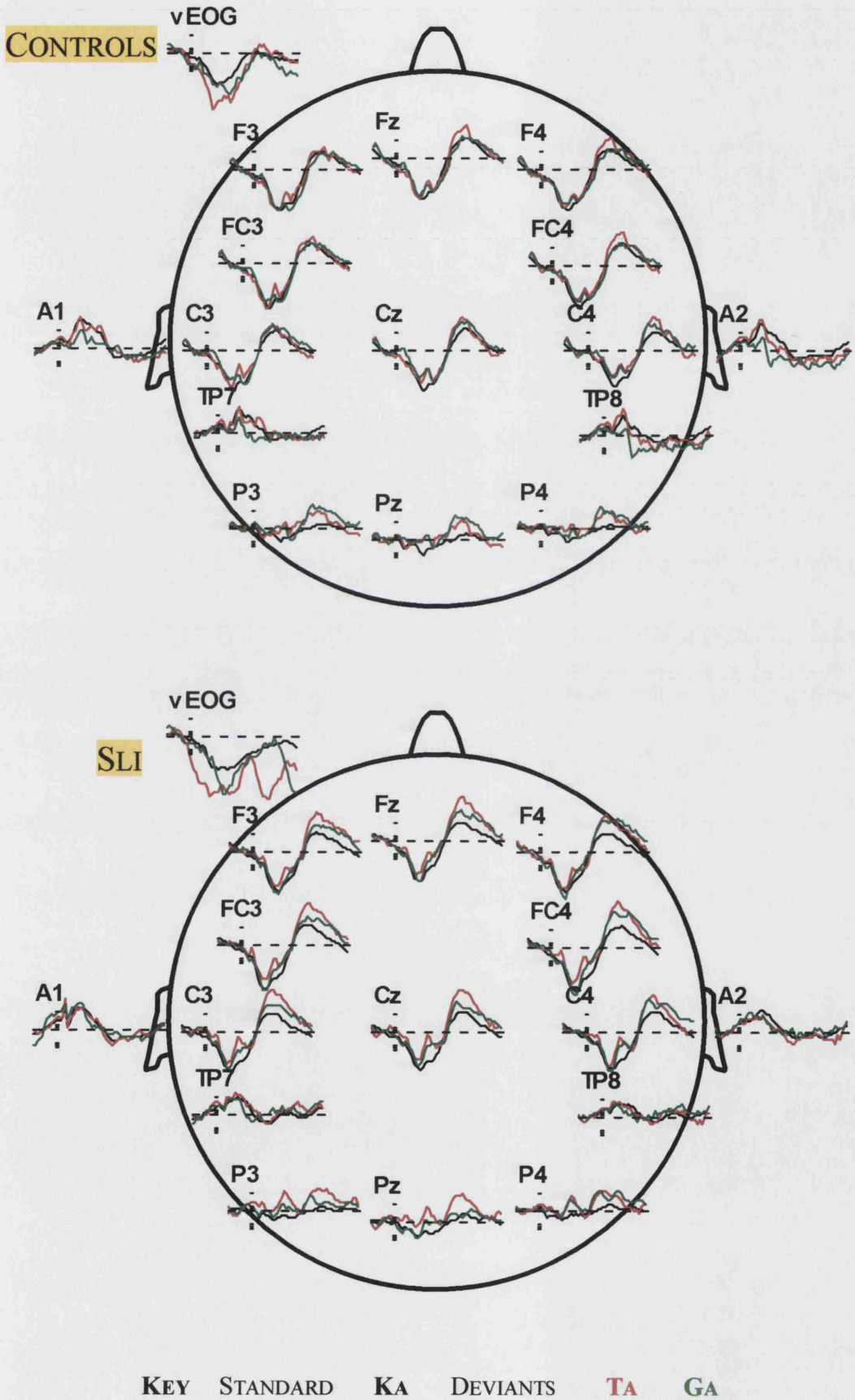
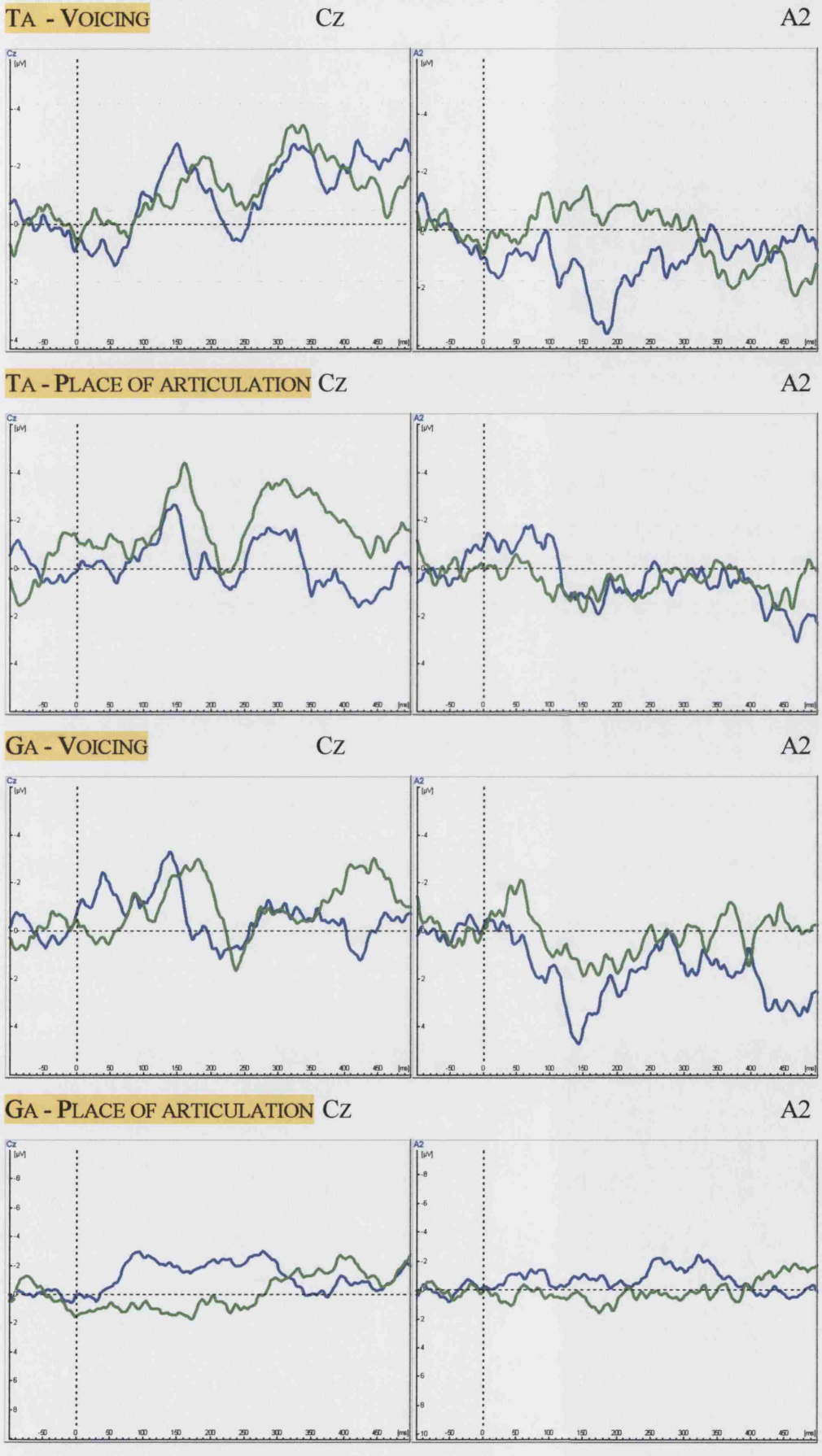


Figure 7.6 Speech MMN (SLI vs. controls)



7.2.3 Relationships between neuropsychological task performance and ERPs in SLI

The SLI-associated pattern of atypical ERPs may reflect contrasting information processing abnormalities implicated in atypical language development. To test this hypothesis, correlational analyses were conducted to assess the relationship between three ERP measures (N1a amplitude, duration MMN latency, voicing MMN mean amplitude) and five measures of cognitive function. The first three neuropsychological measures were chosen because they are hypothetically associated with different aspects of language learning, have been reported as impaired in SLI in one or more studies, and are likely to depend on different (although potentially overlapping or interactive) neural systems. These variables were auditory discrimination of phonemes (the same-different task), verbal working memory (listening span from the Childrens' Working Memory battery; (Gathercole & Pickering, 2000)) and nonword repetition (Gathercole et al., 1994). Two further variables were included as measures of receptive and expressive language attainment:- the British Picture Vocabulary Scale II (Dunn, Dunn, Whetton, & Burley, 1997) and the Repeating Sentences subtest from CELF-III (Semel et al., 1987). This data was collected by Dr Josie Briscoe and Dr Peter Rankin as part of a large battery of neuropsychological assessments completed by the SLI group. An independent sample of control children completed this battery of tests, but was not able to take part in the ERP experiment. This group was matched to the SLI sample on age and nonverbal ability. The SLI children differed from age-matched controls on all measures assessed as potential behavioural correlates of ERP abnormalities (see Table 7.5).

Table 7.6 indicates that good performance on the same-different phoneme discrimination task was associated with small N1a peak amplitudes for both tone and speech standards. There was no significant correlation between these variables in the control group. Table 7.6 also indicates that a relationship exists between the magnitude of the voicing MMN waveform at right temporal electrodes and working memory performance both for words and nonwords (data not available for controls). Unlike the N1a-discrimination relationship, this is a speech-specific association in

that there is no similar relationship between working memory performance and the latency or magnitude of duration MMN.

There were no additional correlations between ERP measures and tests of either receptive (BPVS) or expressive (Repeating Sentences) language performance in the SLI group. This suggests that convergent neural and cognitive anomalies are only risk factors for the development of a language disorder and that other mediating factors may compensate for disruption. Extrinsic factors, especially social and educational environment are highly likely to interact with neurocognitive vulnerability to determine language outcomes such as vocabulary acquisition. Nonverbal ability, or "general intelligence", may be an intrinsic factor that mediates variation in language outcomes in individuals at equivalent neurocognitive risk. To address this hypothesis, partial correlations were computed between BPVS and Repeating Sentences scores, and N1a / MMN measures, however no significant relationships were detected. IQ as covariate

Table 7.5 Cognitive test performance (SLI vs. controls)

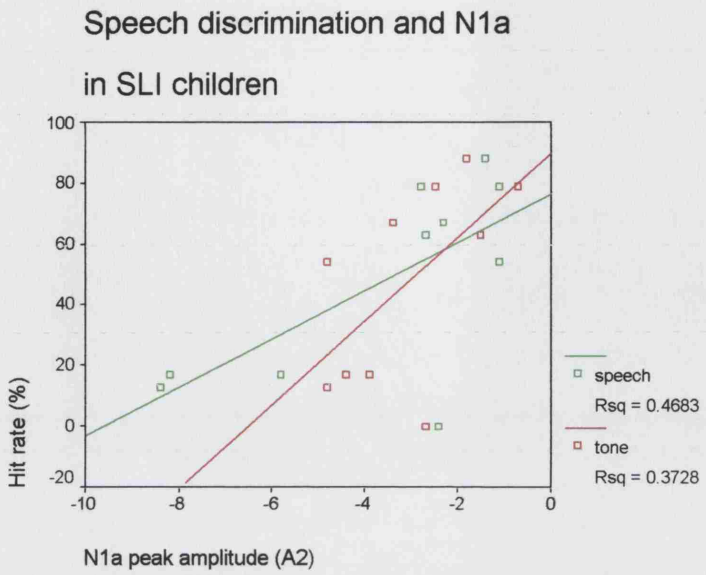
Task	Group		*ANOVA F*
	SLI	Controls	
Speech discrimination task (hit rate)	44 (33)	88 (12)[a]	*16.7* **
Listening span (scaled score)	76 (13)	100 (8)[b]	*22.4* **
CNRep (items correct / 40)	15 (6)	27 (6)[b]	*28.2* **
Repeating sentences (scaled score)	3 (0.4)	11 (3)[b]	*46.4* **
BPVS (scaled score)	86.7 (4.3)	102.1 (9.1)[b]	*26.3* **

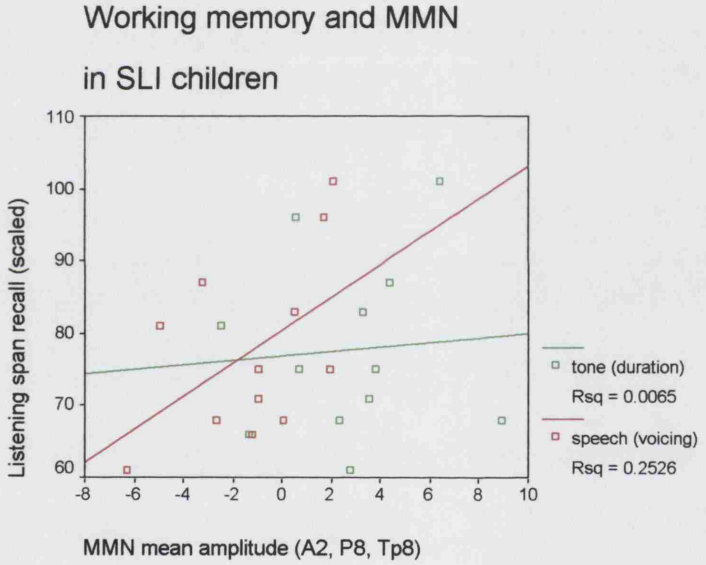
*** p<0.05 ** p<0.01** [a] ERP control sample [b] Non-ERP control sample

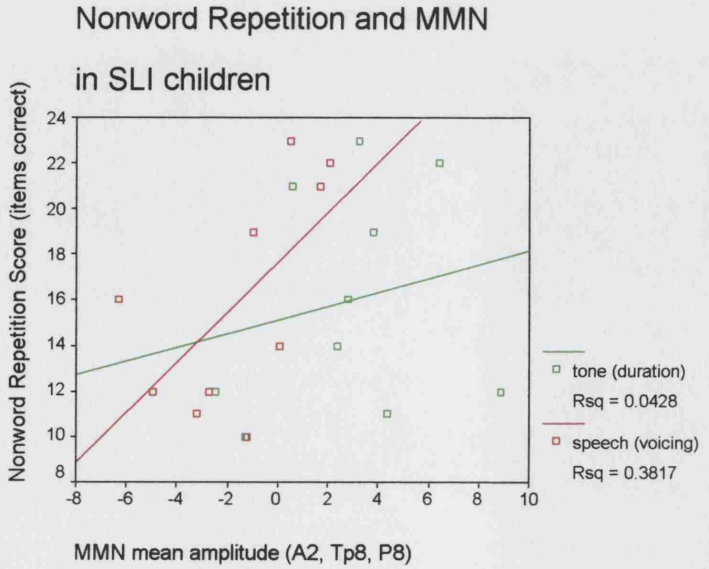
Table 7.6 Relationships between cognitive skills and ERP measures in SLI children

		ERP Component			
		N1a		MMN	
Stimulus		Tone standard	Speech standard	Duration deviant	Ta deviant (voicing)
Measure		amplitude	amplitude	latency	mean amplitude
Electrodes		A2	A2	A1, A2, P8, P7, Tp8, Tp7	A2, P8, Tp8
Cognitive test measure: *Spearman's rho (p-value)*	**Speech discrimination**	***0.64 (0.05)***	***0.55 (0.08)***	*-0.36 (0.3)*	*0.29 (0.38)*
	Listening span	*-0.45 (0.2)*	*0.12 (0.7)*	*0.04 (0.9)*	***0.52 (0.08)***
	Nonword repetition	*-0.05 (0.9)*	*-0.16 (0.7)*	*0.31 (0.4)*	***0.66 (0.04)***
	Repeating Sentences	*-0.20 (0.6)*	*0.28 (0.5)*	*0.06 (0.9)*	*-0.41 (0.3)*
	BPVS	*-0.08 (0.8)*	*0.15 (0.6)*	*0.24 (0.4)*	*0.28 (0.4)*

Figure 7.7 Cognitive-ERP relationships in SLI







7.2.4 Comparison between ERP abnormalities in 22q11DS and SLI

A summary of results from the two case-control ERP studies, alongside developmental data for reference, is presented in Table 7.7. Although both atypical populations (SLI and 22q11DS) showed some abnormalities in both the tone and speech ERP experiments, the pattern of case-control differences (marked in red in table 7.7) was not the same for both groups, and ERP-cognitive relationships (green) were only detected in the SLI group.

Table 7.7 Auditory ERPs - summary of results in typical and atypical development

	Observation	Developmental trajectory	SLI vs. controls	22q11DS vs. controls
Tones	N1 complex	Latency and amplitude decline.	Trend towards amplitude reduction	Amplitude reduced bilaterally
	Frontal MMN	Stable latency and amplitude by age 8.	No difference	Amplitude reduced
	Temporal MMN	Amplitude declines.	Delayed peak latency	No difference
	P3a	Stable latency and amplitude by age 8.	No difference	No difference
	Cognitive correlates		N1 amplitude α speech discrimin.	None detected
Speech	N1 complex	Latency and amplitude decline.	Trend towards amplitude reduction	Amplitude reduced bilaterally
	Frontocentral MMN	Amplitudes stable, increasing context-dependence.	No difference	Amplitude reduced for voicing contrasts.
	Temporal MMN (voicing)	Amplitude declines.	Amplitude reduced	Not apparent
	P3a	Stable amplitude and latency by age 8.	No difference	No difference
	Cognitive correlates		Voicing MMN α working memory	None detected

7.3 *Discussion*

7.3.1 Summary

- On the basis of the experiments conducted in this investigation, children with SLI differ from age-matched control children on three ERP variables.
- Firstly, the amplitude of the obligatory N1 response to repeated stimuli, both for tones and speech sounds, showed a consistent trend towards reduction in the SLI group.
- Secondly, the peak of the MMN response to pure tone deviants (both duration and frequency) recorded at temporal electrodes (but not frontocentral electrodes) was significantly delayed in its post-stimulus latency in the SLI group.
- Thirdly, the amplitude of a prominent right-sided temporal MMN elicited by voicing contrasts between phonemes is absent in the grand average waveforms for the SLI group.
- Thus between-group differences involve both processing of basic auditory features and phonetic contrasts between speech stimuli, and temporal MMN is affected whereas frontal and frontocentral MMN is intact. However the manner in which temporal MMN responses differ between groups is not constant, involving amplitude and latency differences in the different conditions.
- Speech and tone N1a amplitude was associated with speech discrimination ability in SLI children. Although at the group level N1a responses in SLI were smaller than controls, poor discrimination ability was associated with larger N1a. This suggests that poor auditory discrimination in this group is associated with immature processing within the auditory cortex, because N1 amplitudes normally decline with age.
- Temporal speech MMN amplitude was correlated with verbal working memory task performance in the SLI group.

7.3.2 Limitations

- No behavioural test of discrimination of non-speech sounds was carried out in this population (the tone condition of the same-different task was piloted in two SLI children but they found the task difficult to attend to therefore it was

abandoned for remaining subjects). Therefore it is not possible to determine whether the impact of delayed maturation of N1a on auditory discrimination is specific to speech processing or extends to other stimuli and physical features such as duration and pitch.

- Testing of a younger, language-age matched sample might clarify the extent of maturational delay versus developmental difference in the SLI.
- The control subjects for this ERP experiment did not complete the full SLI neuropsychological test battery, therefore the relationship between MMN and working memory in typically-developing children cannot be assessed.
- Small responses generated by the pitch deviant in the tone condition, and /ga/ deviant in the speech condition, makes it difficult to determine the true nature of stimulus-sensitivity of SLI-associated deficits.
- Statistical comparison between the 22q11DS and SLI data was not undertaken because the subject groups were of different ages. It may be of interest to investigate a younger 22q11DS group, and an older SLI group, to determine whether the atypical developmental trajectories in these two populations converge at any developmental time-point.
- Small sample size renders the cognitive-ERP correlations very preliminary. Replication in an independent sample is required, plus extension into larger populations of children with both typical language development and different profiles of speech and language disorder.

7.3.3 Implications

In the study, confirmation of the existence of auditory processing abnormalities in SLI has been obtained. A diverse range of case-control differences were seen at the group level, involving both speech and non-speech sounds, and responses to the standard and deviant stimuli. Uwer et al (2002) reported a speech-specific MMN decrement in children with SLI, which is supported by our data, but only in part. This previous study did not report responses at temporal leads, or in the same time-window, therefore the two reported speech MMN deficits may not correspond to disruptions in the same neural substrate. Korpilahti and colleagues reported a difference in the frontocentral MMN response to frequency change in tones in language disordered children both with and without mental retardation. Since this result has not been replicated either by the current study or by the Uwer et al, and has

also been reported in dyslexia (Baldeweg et al., 1999a) this finding should now be considered a non-specific component of language and literacy disorders, but not a feature of SLI.

Because of the use of nose reference and consequently access to ERPs recorded at the mastoid electrodes, it has been possible in this study for the first time to identify a marked increase in the latency of MMN responses to duration and pitch change in SLI. The observed ~25ms latency shift may be of functional significance given that the temporal difference between the standard and the deviant being parsed here was only 50ms. Delayed processing of a timing difference in the SLI group could also be important for processing of speech sounds, for example voice-onset times, which code the voicing / voiceless contrast within a temporal range of 10-40ms.
This result is interesting in the light of claims that structural changes in the cerebellum, associated with co-ordination of the timing of distributed neural activity, may contribute to risk for language and literacy impairments (Eckert et al., 2003). This results also contrasts with data suggesting that the latency of temporal MMN is reduced in children with autism (Gomot et al., 2002), who sometimes display acoustic hypersensitivity. A reasonable hypothesis, therefore, is that the latency of this ERP component indexes a dimension of pre-attentive sensitivity to sound, which is abnormally high in autism and low in SLI, although the consequences for language development at a behavioural level can appear similar.

Within the SLI population, immature N1 was found to be associated with speech discrimination impairments. This result is broadly consistent with a previous report (Neville, Coffrey, Holcomb, & Tallal, 1993) that N1 deficits are found in SLI children but only is those who performed poorly on Tallal's rapid auditory processing task. This suggests that some aspect of basic auditory stimulus processing, probably carried out within primary or secondary auditory cortex, and not specific to speech sounds, is disrupted in at least some children with SLI. Since the magnitude of the N1 declines with age in normal subjects, this ERP-behavioural relationship indicates delayed maturation in the SLI group in association with auditory processing abnormality. Delayed maturation could be a primary cause of sensory processing deficit, or may indicate the retention of an immature processing system as a secondary consequence of developmental failure elsewhere (perhaps the establishment of adequate phonological representations within long-term memory).

Diminished MMN, recorded at right temporal electrodes in response to voicing contrasts between speech sounds, was found to be associated with poor working memory for verbal material (words and nonwords). Although this relationship has not previously been reported in SLI, it is consistent with the finding that speech MMN is associated with nonword repetition performance in school-aged children (Ceponiene, Service, Kurjenluoma, Cheour, & Naatanen, 1999). This suggests that voicing MMN reflects processing within a neural substrate contributing to the cognitive construct described by Baddeley as the phonological loop. It is important to note that this temporal response to speech deviants declines significantly with age within the normal developmental sample and is absent by mid-adolescence. This suggests a specific developmental function for this neurocognitive circuit. Convergence between ERP and cognitive observations in the SLI group points towards disruption in this neurocognitive circuit as a contributory factor for developmental language disorder.

It has been demonstrated here that perceptual deficits and working memory deficits have distinct neural origins in SLI. Since there was no relationship between these two components (at either the neurophysiological or neuropsychological level) this points towards different pathways towards atypical language development. Heritability of language impairment is high – Dale et al (1998) assessed vocabulary development in 3000 twin-pairs at age 2, and determined that heritability for membership of a language-impaired group, corresponding to the bottom 5 percentiles of the population, was 73%. Estimates for the heritability of the clinical diagnosis of specific language impairment are consistent at around 50%, although these vary somewhat depending on the criteria used for assigning affected-unaffected status (Bishop, 2002). Hence, genetic factors are powerful predictors of language problems, presumably because of the availability of genetic variants impacting upon key neurodevelopmental parameters. The results presented here are consistent with an important study (Bishop et al., 1999) indicating that rapid auditory processing and nonword repetition showed independent heritability in twins affected by SLI. RAP showed equivalent levels of concordance in MZ and DZ twins (implicating environmental factors), whilst NWRep concordance varied as a function of genetic relatedness (implicating genetic factors). This is consistent with the proposal that there are multiple causal and developmental contributions to language impairment

that can be studied more readily by an endophenotypes approach than via categorical diagnoses. The current study has confirmed the independence of perceptual and working memory deficits at the neurophysiological level. Therefore these measures may be valid endophenotypes for investigation of the impact of candidate genetic polymorphisms on neurocognitive risk factors within the SLI population. Study of the siblings of children with SLI using neurophysiological measures would address the question of whether ERP abnormalities are endophenotypes for language disorder or consequences of atypical development.

It has been shown in this study that neurophysiological tools are powerful for dissociating between patterns of atypical development within a group selected for homogeneous behavioural abnormality (in this case phonological-syntactic SLI). Convergence between ERP and cognitive measures has identified potentially distinct pathways towards atypical language development. Further work should explore the properties of these pathways at different points during development, in both typical and atypical language-learners. Combining these measures with MRI methodology will determine the neuroanatomical basis for these distinct patterns of impairment, and functional MRI may further elucidate the neural circuits and their activity during language-processing and language-learning tasks. Comparisons between SLI children and other children with compromised language development (for example pragmatic disorders) would determine whether disruption to the pathways identified can result in contrasting linguistic profiles. Study of children with mild hearing impairment and dysphasias would indicate peripheral-central interactions in mechanisms of language development.

The contrast between atypical ERP profiles in 22q11DS and in SLI confirms that there is more than one pattern of possible disruption to the neural circuits underlying auditory oddball ERPs. Moreover, there is no profile that is characteristic of all groups with developmental language disorders, given that language impairment is found in both of these populations. Certain aspects of the contrasting profiles are particularly noteworthy. First, the topographical dissociation (frontocentral abnormalities in 22q11DS, temporal abnormalities in SLI) confirms yet again that these two ERP sites are not reflections of the same ERP generator. Further work is required to increase our understanding of the neuroanatomical generators, neurochemical modulation and functional / developmental significance of each

component. Second, the altered pattern of stimulus-sensitivity (predominantly speech MMN disruption in SLI, speech and tone MMN disruptions in 22q11DS) further indicates the selectivity of deficits and points towards at least partial independence of speech and non-speech processing. Lastly, distinct ERP-cognitive correlates (relationships between ERPs and speech discrimination, working memory and language function were not found in SLI and not in 22q11DS) indicate that disruption to these systems can have diverse consequences on cognitive development.

8 Investigating a potential genetic mediator of neuropsychiatric risk in 22q11DS

8.1 Introduction

8.1.1 Overview

In previous chapters, evidence for the presence of neurocognitive impairments and psychological indicators of neuropsychiatric risk in 22q11DS has been presented. Adolescents with 22q11DS show schizophrenia-like deficits in auditory processing and working memory, and individuals with more severe neurocognitive disruption are also more likely to display schizotypal features. There is no clear categorical distinction that can be drawn between low and high-risk individuals, at least on the basis of the data collected here, thus a continuum of risk-related disruption seems most accurately to characterise the population. This continuum of risk, as reflected in endophenotypic features, suggests that haploinsufficiency of a key gene within the 22q11 region renders a specific developmental or functional process vulnerable and that variation in the impact of haploinsufficiency is mediated by additional individual differences within the 22q11DS population. These individual differences may be of either genetic or environmental origin, and may either compensate for, or potentiate, vulnerability of the processes underlying psychiatric risk.

Modifying influences could be either proximal or distal to the causative gene. Proximal influences, for example, could be individual differences within the single available copy of the causative gene, or in upstream regulators or downstream targets of the gene in question. Distal influences modifying degree of vulnerability may exist as a consequence of embryological, neural or cognitive redundancy, impacting upon the individual's capacity to compensate for haploinsufficiency, plus the multitude of chaotic environmental factors impacting upon every aspect of development.

The hypothesis to be tested in this chapter concerns a potential proximal influence on the continuum of risk within 22q11DS. Individual differences in the single copy of a candidate gene within the typically deleted region of 22q11 may modify the

expression of endophenotypic features. Evidence that variation in a critical genetic factor influences an endophenotype within the 22q11DS population would only indirectly suggest that haploinsufficiency of this gene is causally responsible for increased neuropsychiatric risk in this population. However by associating variation in a gene of known functional significance with the continuum of neuropsychiatric risk, physiological mechanisms underlying vulnerability could then be explored.

8.1.2 COMT: history, genetics, biochemistry and neural circuitry

The 22q11 typically deleted region harbours a well-characterised gene, *catechol-o-methyl transferase* or *COMT*. *COMT* has long been implicated in risk for psychiatric illness, as a consequence of linkage studies and functional candidacy, and has probably been the subject of more association studies than any other single gene in psychiatric genetics. By fortuitous circumstance, a functional variant in this gene has in recent years been the subject of very intensive investigation. Not only have studies examined the significance of this gene and its functional variant for risk of psychiatric disorder, but as a consequence of harnessing the endophenotypes approach and integrating findings from diverse methodologies, potential neurobiological and cognitive significance has been illuminated. Current knowledge of *COMT* structure and expression, its impact upon catecholamine regulation in the brain, and consequently on cognitive function and psychiatric illness is outlined below. This emerging knowledge may assist in elucidating mechanisms of neuropsychiatric risk in 22q11DS, and any evidence of a role for *COMT* variation in mediating risk in 22q11DS would, in a reciprocal fashion, contribute to the emerging model of *COMT*-influenced neurocognitive function.

Catechol-O-methyltransferase is an enzyme that catalyses the transfer of the methyl group of S-adenosyl-L-methionine to a phenol group, inactivating the catchol substrate of many biologically active amines including dopamine, noradrenaline and adrenaline (Axelrod & Tomchick, 1958). Pre-genetic studies of enzymatic activity assayed from peripheral blood indicated that there is considerable inter-individual variation in COMT enzyme activity. About a quarter of the Caucasian population express a heat labile form of the enzyme associated with approximately four-fold reduction in activity (Weinshilboum & Raymond, 1977). This individual difference in enzyme activity levels was shown to be heritable by Grunhaus and colleagues

(1976) in a monozygotic-dizygotic twin study. Interest in individual differences that could contribute to variation in catecholamine metabolism, potentially of significance for psychiatric disorder and responses to medication, resulted in several early attempts to measure enzyme levels in patient populations including schizophrenia and affective disorders but with equivocal results. The *COMT* gene was mapped to its chromosomal location at 22q11 (Grossman, Emanuel, & Budarf, 1992), and the genetic variant underlying individual differences in enzymatic activity was identified by Lachman et al (1996). A G → A substitution at codon 158 in the genomic sequence for the membrane-bound form of the enzyme (or codon 108 of the shorter transcript corresponding to the soluble form) results in a valine to methionine amino acid substitution in the peptide sequence. *COMT158*^{val} genotype corresponds to the high-activity form of the enzyme and *COMT158*^{met} to the low-activity form.

COMT is expressed widely in both the body and brain, but its functional significance may be regionally specific because of the absence in certain structures of additional catabolic enzymes, in particular monoamine oxidase (MAO-A and MAO-B), and of synaptic uptake mechanisms. In the brain, COMT may be a limiting factor for removal of catecholamine neurotransmitters in the prefrontal cortex where uptake transporters are not heavily expressed (Lewis et al., 2001). Membrane-bound COMT is localised to post-synaptic dendrites and astrocytic processes surrounding catecholaminergic synapses. A recent study in *COMT* knockout mice (Huotari et al., 2002) showed that, under basal conditions, COMT enzyme variation may be insignificant in regulating transmitter availability. However, under certain acute challenges (for example a surge of levodopa administered by microdialysis), enzyme deficiency in both homozygous knockouts and heterozygotes resulted in rapid accumulation of dopamine and oxidised metabolites in brain homogenate.

The relationship between genotype and enzyme activity levels was established in peripheral blood, and caution should taken in assuming that the same relationship exists between *COMT*^{val}*158*^{met} genotype and enzyme activity levels in the brain, where additional regulatory influences may exist. An important study (Akil et al., 2003) recently extended our understanding of the impact of *COMT* variation on human brain function, by measuring tyrosine hydroxylase (TH) mRNA (a rate-limiting enzyme for dopamine synthesis) in mesencephalic dopamine neurones. These dopaminergic cells receive indirect projections from pyramidal cells in the

prefrontal cortex and project both back to the frontal cortex and on to the striatum and limbic structures. This may be necessary for the maintenance of optimal signal-to-noise ratio for information processing within the prefrontal cortex. Analysis of post-mortem TH levels in the substantia nigra revealed that *COMT*$^{val/val}$ individuals have significantly higher levels of the synthetic enzyme in dopaminergic neurones than do *COMT*$^{val/met}$ individuals. This leads to the speculation that COMT is integrated within a feedback system for minimising resting dopamine levels in the prefrontal cortex and in other areas of the brain. Individual differences in *COMT* genotype and enzyme activity could therefore have quite widespread feed-forward impact on neurotransmitter regulation and cortical function during development and adult life.

8.1.3 *COMT*^{met}*158*^{val} - a susceptibility factor or modifying influence in psychiatric disorders

A recent meta-analysis of case-control and family-based association studies (Glatt, Faraone, & Tsuang, 2003) indicated that the *COMT*^{met}*158*^{val} polymorphism accounts for at best a small proportion of population variance in schizophrenia risk, in Caucasian populations only. Pooled results from 14 case-control studies failed to detect an overall significant effect of genotype on risk, with a very weak trend towards increased risk in association with *COMT158*^{val} (odds ratio = 1.1) and two studies finding significantly increased frequency of the *COMT158*^{met} allele in the patient group. 2 of 5 family-based studies (assessing transmission disequilibrium of genotypes from unaffected parents to affected versus unaffected offspring, the so-called TDT method) did detect a significant effect, with the *COMT158*^{val} allele conferring increased risk with a pooled odds ratio of 2.2 (95% C.I.=1.4 – 3.4). Of note is the fact that these two studies were of European populations whilst the remainder were of Asian origin. The discrepancy between results generated by the two association methods, and between studies involving different ethnic populations, indicates that these methodologies are very prone to error on the basis of sample differences in allele frequencies.

After this meta-analysis was conducted, Shifman et al (2002) published a haplotype association study examining 12 SNPs (single nucleotide polymorphisms) within *COMT* in a population with low genetic variance (Ashkenazi Jews). A highly

significant association was found between schizophrenia and a three-marker haplotype that includes the *COMT158^{val}* SNP in association with specific variants of neighbouring markers. Previous single SNP studies may have failed to detect consistent effects of genotype because the key polymorphism is a nearby SNP in linkage disequilibrium with *COMT^{met}158^{val}* or because risk is associated with the specific combination of SNPs identified in this haplotype. Thus although it seems likely on the basis of this study that the *COMT* gene is implicated in risk for schizophrenia, there may be multiple significant variants, potentially varying in different ethnic populations. It is also noteworthy that this study found different degrees of association between risk haplotypes and schizophrenia in males and females, indicating the possibility of sex-specific interactions between *COMT* and other risk factors. A follow-up study (Bray et al., 2003) of quantitative mRNA transcription levels in post-mortem tissue of 60 normal humans (drawn from control brain banks used in previous psychiatric studies), indicated that the risk haplotype found by Shifman et al (2002) was associated with reduced expression of the *COMT* gene, providing further evidence for auto-regulatory feedback as shown by Akil et al. Thus the presumption that high loading for the *COMT158^{val}* allele results in constitutively higher rates of dopamine clearance in the frontal cortex may not hold true, at least in the context of the schizophrenia-risk haplotype.

The same functional polymorphism within *COMT* has been investigated as a possible risk factor for other psychiatric disorders in addition to schizophrenia. Craddock et al (2001) reviewed case-control association studies in bipolar disorder for a number of different candidate genes, including *COMT.* There is considerable reason to believe that schizophrenia and bipolar disorder share some genetic risk factors; the illnesses have a degree of phenotypic overlap (psychosis, affective disruption), may cluster together within families, and linkage studies have indicated some common areas of genomic disequilibrium (Berrettini, 2000). Craddock et al combined association data from seven studies seeking an association between *COMT^{met}158^{val}* and bipolar disorder, finding a pooled odds ratio of 1.18 (C.I.= 1.02 – 1.35), suggestive of a very weak effect of the low activity allele on increased susceptibility for this diagnosis.

An additional possibility is that the *COMT* variant could be a modifier of the course of bipolar illness. Kirov et al (1998) found that the *COMT158^{met}* allele was

significantly associated with rapid cycling versus non-rapid cycling illness (defined as at least one year during which four episodes occur, demarcated by either remission or swing to opposite polarity). In this study, allele frequencies did not differ between the whole patient sample and the control population, but there was a dose-dependent relationship between the low-activity allele and likelihood of rapid cycling given that one had a diagnosis of bipolar disorder. Similar evidence was found in a sample of child and adolescent-onset bipolar cases, in whom presence of an ultra-ultra rapid cycling illness was again associated with the low-activity allele (Papolos, Veit, Faedda, Saito, & Lachman, 1998). All but one of six patients with this diagnosis were homozygous for *COMT158^{met}*. One possible explanation for this association is that rapid cycling is more common in females than males (although overall prevalence of bipolar disorder is not gender-biased). This suggests a gender-specific association between *COMT* allele and cycle frequency, potentially in connection with hormonal cycles which may stress the "buffering" capacity of neurochemical systems. A second proposal put forward by Papolos is that induction of rapid cycling mood disorder by anti-depressant medication could be of higher likelihood in individuals with low COMT activity. This could be particularly significant during childhood, when prescription of medication to vulnerable individuals might be more likely to precipitate a prolonged and severe disturbance of neurochemistry than in adults whose homeostatic mechanisms might be more stable. On the other hand, the reported associations with cycling could represent an association with severity of neurobiological disruption implicated in mood disorder, particularly in early-onset cases.

A similar modifying influence of the *COMT^{met}158^{val}* polymorphism has been reported with respect to aggressive behaviour in schizophrenia, although results have been inconsistent. Review of the existing literature on this subject (Strous et al., 2003) identified four studies that have found an association between the low-activity allele and violence or aggression in patients with schizophrenia. Two studies failed to detect an association, and one study found a significant association between aggression and the high-activity allele. Definitions of aggression vary markedly in these studies, from homicidal offending to suicidality to verbal aggression against psychiatric staff. Studies have on the whole categorised patients as either violent or non-violent, whereas in fact behaviours classed as violent or aggressive are likely to vary quantitatively along several different and potentially unrelated dimensions such

as impulsivity, depressive aggression, self-harm and psychosis-related violence. Strous et al (2003) presented a study of their own, rating aggression on several scales derived from an interview inquiring about many different aspects of behaviour in a sample of 122 inpatients with schizophrenia. A significant effect of *COMT158*^{met} was found for dimensional scores relating to aggression and self-directed violence but not anti-social behaviour, whilst no similar effects were found for MAOA, another potential regulator of catecholamine function. Possible explanations for this finding are similar to those proposed for the modifying influence of *COMT158*^{met} on cycling in bipolar disorder. The genotype-phenotype association could reflect a specific subtype within the schizophrenia spectrum, with the low and high activity alleles biasing dopamine function in the frontal cortex in different directions, precipitating some similar symptomatology but having alternative effects on cognition and impulsive-aggressive behaviours. An alternative or possibly additive mechanism could be via contrasting genotype-influenced responses to anti-psychotic medication, or psychosocial stressors, precipitating different behavioural consequences.

Lastly there is evidence, though again inconsistent, of a gender-modified (males only) association between the *COMT158*^{met} allele and obsessive compulsive disorder (Karayiorgou et al., 1999). Obsessive thoughts (sometimes difficult to dissociate from delusions) and repetitive or compulsive behaviours are also seen in patients with schizophrenia, with a recent prospective study of 150 male patients detecting these features in approximately 10% of cases during an acute psychotic episode (Fabisch, Fabisch, Langs, Huber, & Zapotoczky, 2001). A dependence of these symptoms on a specific pattern of neurocognitive disruption was recently suggested by Lysaker et al, (2003) who found that schizophrenia patients with obsessive-compulsive features performed worse than non-obsessive patients on tests of executive function, but better on tests of visual memory. OCD also involves frontostriatal disruption (Rosenberg & Keshavan, 1998), suggesting that *COMT*-influenced catecholamine dysfunction could precipitate diverse symptoms that are dependent on similar neural circuitry. One prediction yet to be tested would be that the subset of schizophrenia patients with obsessive-compulsive symptoms and associated cognitive deficits are more likely to harbour the low-activity *COMT158*^{met} allele at high frequency.

In summary, there is only marginal evidence that the *COMT*^{met}*158*^{val} polymorphism selectively influences susceptibility to any one particular psychiatric illness. Given the weak associations reported between genotype and schizophrenia, bipolar disorder and OCD, it seems more plausible that *COMT* variants can predispose a subset of individuals, who also harbour other risk factors, to any one of a range of categorical diagnoses. Existing reports of within-diagnosis impact of *COMT*, on cycle frequency in bipolar disorder and aggression in schizophrenia, emphasise the heterogeneity within each of these diagnostic groups. However no study has yet examined commonality across diagnostic groups in symptoms or neural systems influenced by *COMT*. The relatively consistent impact of the *COMT158*^{met} allele, raising the likelihood of particularly poor outcomes in both schizophrenia and bipolar disorder, suggests that a common mechanism may modify prognosis given the presence of either disorder. The impact of *COMT* variant may act across-diagnoses to modify a neurobiological parameter in such a way as to predispose key neurocognitive systems to dysfunction.

8.1.4 *COMT* and neurocognitive markers of psychiatric risk

Recent studies have addressed this possibility by associating cognitive function, rather than diagnoses, with *COMT*^{met}*158*^{val} genotype in both schizophrenia patients and the general population. Egan et al (2001) conducted a highly influential study to this end, assessing the impact of *COMT* genotype on Wisconsin Card Sorting Test (WCST) performance in patients, healthy siblings and unrelated controls. The WCST is a well-known test of executive function, reliant upon logical reasoning, cognitive flexibility and working memory, found in many studies to be a reliable deficit in schizophrenia (Goldberg & Weinberger, 1988). In addition, WCST performance is poor in high-risk offspring of schizophrenia patients whether or not psychosis emerges, suggesting that it could be an endophenotype for schizophrenia-related neurodevelopmental vulnerability (Wolf, Cornblatt, Roberts, Shapiro, & Erlenmeyer-Kimling, 2002). Results in affected/unaffected twin pairs and in adult relatives have been more equivocal (Laurent et al., 2001), reflecting population characteristics (general cognitive ability and schizotypal personality in relatives may be important modifiers), and different indices used to report performance (e.g. perseverative errors, number of categories achieved, number of trials to completion of first category).

Egan et al (2001) found that both patients and healthy siblings made more WCST perseverative errors than controls, and moreover that performance was dependent on genotype in all three groups. The *COMT*$^{val/val}$ genotype was associated with worst performance, and a parametric relationship was found to exist between loading for the *COMT158*^{met} allele and improved performance (albeit accounting for only 4% of variance in performance). By including diagnosis, age, gender and educational level in the stepwise regression, the significance of genotype as a predictor of performance was retained. Replication of this result was obtained in relatives (Joober et al., 2002) and in the general population (Malhotra et al., 2002), and broadly similar results (association between *COMT158*^{met} and improved processing speed and attention) in chronic schizophrenia patients (Bilder et al., 2002). These studies provide consistent evidence that *COMT* genotype influences cognitive functions thought to be dependent on prefrontal cortex and associated with increased risk for schizophrenia regardless of the illness-status or risk-status of the population investigated.

Egan et al (2001) also addressed the possible neural basis for cognitive variation found in association with *COMT* genotype. Functional MRI data was presented alongside the WCST results, although only for siblings, showing that *COMT*$^{val/val}$ genotype is associated with more widespread activation of the dorsolateral prefrontal cortex and cingulate for the same level of performance during an N-back working memory task. This effect of genotype on areas of activation is interpreted as an indication of inefficient processing in these cortical structures. In *COMT*$^{val/val}$ individuals inefficient processing may be a consequence of reduced signal-to-noise ratio secondary to reduced synaptic availability of dopamine.

A neurocognitive model currently under investigation is that dopaminergic function in the frontal cortex does not follow a linear function-performance relationship, but conforms to an inverted U-shaped curve, with processing compromised when dopamine availability and D_1 receptor occupancy is either too high or too low (Goldman-Rakic, Muly, III, & Williams, 2000). This model is generating testable hypotheses that may explain contradictions within the psychiatric and general cognitive literature on *COMT*. In primates, the optimal-dopamine effect has been shown for spatial working memory function, assayed by an ocular delayed response task that requires effortful fixation on a central spot then movement towards the

location of a memorised target spot. At very low levels of D_1 receptor occupancy, loss of dopaminergic facilitation of glutamatergic inputs from visual areas to the prefrontal cortex prevents the establishment of spatially-tuned memory fields. Memorisation of ODR target locations is optimised by applying D_1 agonists, but then declines on administration of amphetamine, or, interestingly, by applying an acute behavioural stressor to the awake primate (Williams & Goldman-Rakic, 1995).

The significance of appropriate dopaminergic function for working memory performance in schizophrenia was demonstrated by Abi-Dargham et al (2002). In this study, D_1 receptor occupancy was measured with positron emission tomography and the selective D_1 receptor antagonist [^{11}C]NNC112, in patients (either drug-naïve or drug-free at the time of the experiment) and controls. Binding potential for the tracer was significantly increased in the dorsolateral prefrontal cortex of patients, indicating underactivation by endogenous dopamine and suggestive of compensatory increases in receptor expression. Moreover, low receptor occupancy (high binding potential) was linearly associated with poor performance in the N-back working memory task, indicating the functional significance of reductions in dopaminergic function. This result converges with Egan et al's finding that the high-activity enzyme and low dopamine transmission is association with impaired cognitive function.

Regulation of dopamine flux by *COMT* could be important in maintaining optimal performance in the face of either reductions or surges in input. Thus both high and low activity alleles could be advantageous or deleterious under different challenging circumstances. Evidence for this was recently provided by Mattay et al (2003), who challenged normal adult volunteers with amphetamine or placebo in a double-blind N-back fMRI experiment. At placebo baseline $COMT^{met/met}$ participants performed at a superior level to $COMT^{val/val,,}$ but following amphetamine dose, working memory performance declined in the former group whilst improving in the latter. Performance increases were mirrored by reduced intensity of prefrontal activation. This suggests that baseline dopamine signalling was optimal in $COMT^{met/met}$ participants, but that blockade of uptake mechanisms by amphetamine results in a hyperdopaminergic state that reduced processing efficiency. Individuals harbouring alleles for the high activity enzyme, on the other hand, moved from a relatively hypodopaminergic state at baseline to a functionally more advantageous state

following amphetamine administration. Interestingly, heterozygous individuals did not display changes in executive function or working memory performance following drug administration, although they did show reductions in prefrontal activation, suggesting that this may be the most homeostatically stable genotype, perhaps explaining the maintenance of both genotypes at high frequency within the general population. Future work on this system should address the longer-term implications of genotype on regulation of resting and dynamic dopamine flux, for example on expression of receptors and uptake transporters.

In conclusion, many studies have sought and found evidence for an influence of *COMT* on susceptibility to psychiatric disorder, and on cognitive and neural function that may contribute to disorder, with results that are both consistent and confusing. On the one hand *COMT158* ^{val} is associated with both risk for schizophrenia and schizophrenia-related cognitive impairment, possibly via a prefrontal hypodopaminergic state. On the other hand *COMT158* ^{met} is associated with risk for other psychiatric disorders (bipolar and OCD) and for aggression in the context of schizophrenia, also suggestive of prefrontal or frontostriatal dysfunction. This allele is also associated with cognitive dysfunction, but only under certain circumstances (amphetamine challenge, as shown by Mattay et al, and in the context of schizophrenia as shown by Gallinat et al in a P300 study, see below). Investigations of potential neural correlates of variant genotypes are suggestive of an impact of enzyme variation on efficiency of prefrontal cortex activation but further experiments are required to clarify interactions within this undoubtedly complex system.

8.1.5 Mismatch negativity as an assay of dopaminergic efficiency in the prefrontal cortex

Evidence that dopamine exerts a modulating influence on auditory attentional processing has been obtained via a small number of investigations of the impact of dopamine blockade on auditory ERPs in normal adult subjects. Kahkonen et al (2001) conducted a randomised double-blind placebo-controlled crossover study of the effects of haloperidol on cortical activation during a dichotic listening task using MEG and high-density EEG. Following drug administration, and thus dopamine receptor blockade, the processing negativity associated with task-relevant (to be

attended) tones was attenuated, whilst MMN elicited by task-irrelevant (to be ignored) tones was enhanced. Pekkonen et al (2002) showed that the magnetic MMN to frequency change was accelerated (shorter latency) by haloperidol relative to placebo, again indicating that memory-based comparison processes are facilitated by reducing the availability of dopamine. In contrast, later P3a and so-called reorienting negativity (RON) were attenuated by haloperidol, indicating the possibility of a dopamine-mediated trade-off between the processing of distracting information (MMN), and facilitation of focused attention (P3a / RON) (Kahkonen et al., 2002). In addition to dopamine, COMT acts on other important substrates (serotonin, noradrenaline) which may modulate some aspects of auditory change detection but which have not yet been investigated via pharmacological manipulations.

One strategy for enhancing current understanding of dopaminergic influences on auditory attention would be to assess the impact of the functional *COMT* polymorphism on ERPs. This has not yet been carried out with respect to MMN, but two studies have addressed the potential impact of $COMT^{met}158^{val}$ genotype on the later P3 component. A marginal association between $COMT^{met/met}$ genotype and faster P3 elicitation (shorter latency) in normal female subjects, suggesting more efficient prefrontal function in individuals with the low enzyme activity and (presumed) higher resting dopamine levels (Tsai et al., 2003). A contradictory report from Gallinat et al (2003) suggested that the $COMT^{met/met}$ genotype was associated with smaller P3 amplitudes, an effect that was significant in schizophrenia patients but not in healthy controls. This result is more in line with evidence presented above that dopamine is an inhibitory influence on pre-attentive processing. It also suggests that genotype could modify neural function differentially dependent on diagnosis, presumably via interactions with other genes and chronic differences in neural functioning and dopamine availability.

It is hypothesised that in 22q11DS, *COMT* haploinsuffiency may result in elevated resting dopamine levels and reduced signal-to-noise ratio in the frontal cortex, explaining impaired mismatch negativity elicitation. If this is the case then variation within the 22q11DS population in the ability to compensate for reduced dopamine clearance might impact upon neurocognitive processing, as reflected by MMN.

8.1.6 *COMT* in 22q11DS

There is little direct evidence at present to support the view that *COMT* haploinsufficiency contributes significantly to neuropsychiatric risk in 22q11DS. Graf et al (2001) studied four adolescent patients with 22q11DS, all harbouring the low activity *COMT158*^{met} allele, and all displaying a range of severe psychiatric symptoms that included affective "storming" and psychosis. The investigators instituted an open-label trial of metyrosine, a competitive inhibitor of tyrosine hydroxylase, in an attempt to reduce catecholamine production and normalise prefrontal transmission. This resulted in subjective improvements in symptoms (although not in objective neuropsychological performance, tested in only two cases), alongside falls in blood and urine catecholamine and metabolite concentrations (to within the reference range from mildly elevated pre-treatment levels). No data was provided on the long-term maintenance of these effects, nor on any differential impact of this treatment in 22q11DS relative to that expected in any individual displaying marked psychiatric symptoms, nor on the impact of metyrosine on similar symptoms in 22q11DS individuals with the high-activity *COMT158*^{val} allele. A systematic, double-blind study controlling both for 22q11DS-status and *COMT* genotype would address these issues.

Inferential evidence for the significance of *COMT* haploinsufficiency would be achieved if an additional impact of *COMT*^{met}*158*^{val} genotype on cognitive or psychiatric functioning in 22q11DS could be detected. A general hypothesis is that *COMT* insufficiency renders this homeostatic system brittle to disruptions, i.e. less efficient at auto-regulating in the face of deviations from a central tendency, and more likely to swing from one extreme to another, or to exist in a stable but overactive or underactive state. Given the evidence that *COMT*$^{met/met}$ individuals are at a cognitive advantage at baseline one might predict that under stable conditions 22q11DS individuals may be able to maintain relatively normal dopamine flux. However in situations when dopaminergic input is increased (as mimicked by amphetamine administration in the Mattay et al study), capacity to clear dopamine from synapses and maintain optimal signal-to-noise ratio would be exceeded and cognitive performance would be expected to decline.

No strong predictions can be made regarding any additional impact that the *COMT*^{val}*158*^{met} genotype, present on the intact chromosome 22 in 22q11DS, may

have on regulating neurochemical function and influencing cognitive and psychiatric risk. However one allele could putatively be more able to compensate for haploinsufficiency than the other. 22q11DS individuals harbouring a *COMT158*^{met} allele would have very little capacity to buffer dopamine increases. However Egan et al's finding, replicated by Mattay et al, of reduced baseline efficiency of the *COMT158*^{val} allele in regulating prefrontal activity, would suggest that 22q11DS plus *COMT158*^{val} allele could be more disadvantageous. Finally, evidence from Bray et al that transcriptional suppression of the *COMT158*^{val} allele may compensate for higher enzymatic activity levels, plus evidence from Akil et al of COMT-influenced feedback from prefrontal neurones to dopamine-producing cells in the midbrain, suggests that compensatory mechanisms could result in no difference between the genotypes over and above the potential impact of haploinsufficiency.

Two previous studies have addressed the possible impact of the lone *COMT*^{met}*158*^{val} genotype on the expression of psychiatric illness in 22q11DS. Papolos et al. (1996) found that the *COMT158*^{met} allele was not associated with the presence or absence of any particular disorder, but was associated with higher likelihood of rapid cycling course in patients with 22q11DS and bipolar disorder. This was established in a sample of 23 children and adolescents, 21 of whom met criteria for at least one psychiatric disorder. Papolos therefore concluded that the *COMT158*^{met} allele has the same impact on the course of affective illness in 22q11DS as it does in children and adolescents from the general population with similar symptoms. However, it should be noted that several subjects in this study displayed a bipolar illness that was rapid cycling (although not ultradian) in the context of 22q11DS-*COMT158*^{val}. Additionally, no other study has reported such high rates of bipolar disorder in 22q11DS, suggesting that either the population studied by Papolos, or his method of assessment and assignment of diagnoses, may be unique. Murphy, Jones, and Owen (1999) typed the *COMT* polymorphism in their adult sample of 40 individuals with 22q11DS, of whom 30% were diagnosed with schizophrenia. No association was found between schizophrenia diagnosis and *COMT* allele, nor between *COMT* allele and scores on the King's Schizotypy Questionnaire. This suggests that there is no impact of the enzyme variant on likelihood of developing schizophrenia or of displaying schizophrenia-spectrum features in the context of 22q11DS. However it may be that the allele modifies specific symptom dimensions (such as affective instability, not addressed within the schizotypy construct) or illness parameters (such

as age-of-onset, chronicity or medication response) which were not addressed in the Murphy et al study.

8.1.7 Summary

These is some very limited evidence (from Graf et al 2001) that dopaminergic dysregulation impacts on the psychiatric phenotype of 22q11DS, but no evidence that *COMT* genotype influences susceptibility to schizophrenia or other diagnoses and only some very weak evidence that genotype may modify prognosis of psychiatric disruption in 22q11DS. However it is theoretically plausible that dopaminergic dysregulation is involved in symptom generation and evolution of psychiatric illness in 22q11DS. It is proposed that evidence of dysregulation occurring as a consequence of *COMT* haploinsufficiency is more likely to be detectable in the form of a neurocognitive endophenotype than a diagnosis or symptom. The hypothesis tested here is that $COMT^{met}158^{val}$ genotype modifies expression of neurocognitive endophenotypes in 22q11DS adolescents, in a manner that indicates vulnerable homeostatic regulation of dopamine levels in the prefrontal cortex.

8.2 Method

Buccal swabs were dispatched by post to every participant in this study (22q11DS and controls), and to their biological parents where available. Genotypes were established in controls in order to obtain an estimate of allele frequency in the general population, and in parents to account for potential ethnic differences in allele frequency.

The DNA was extracted from buccal swabs using the QIAamp DNA Mini Kit (QIAGEN). The recommended protocol was followed. DNA was eluted in 150ul of elution buffer (supplied with kit). The $COMT^{met}158^{val}$ polymorphism was then amplified using two primer pairs.

Primer pair 1:	Forward	TCA CCA TCG AGA TCA ACC CC
	Reverse	GAA CGT GGT TGT AAC ACC TG
Primer pair 2:	Forward	AGC TCC AAG CGC GCT CAC AG
	Reverse	TGG GTT TTC AGT GAA CGT GG

PCR was performed using primer pair 1 in a total reaction volume of 25µl using the PuReTaq™Ready to Go™ PCR beads (Amersham Biosciences). Forward and Reverse primers were added to a final concentration of 0.4µM. The product was amplified using the following cycling condition: 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, 62°C for 30 seconds and 72°C for 30 seconds, and a final extension at 72°C for 2 minutes. Amplified product of 188bp was electrophoresed in a 1.5% agarose gel and visualised with ethidium bromide.

PCR was performed using primer pair 2 in a total reaction volume of 50µl that contained approximately 100ng of genomic DNA, 1x NH_4 Bioline buffer, 1mM $MgCl_2$, 0.05mM dNTP, 0.2µM forward and reverse primer and 2.5U BioTaq polymerase. The product was amplified using the following cycling condition: 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for 30 seconds, and a final extension at 72°C foe 2 minutes. Amplified product of 532bp was electrophoresed in a 1.5% agarose gel and visualised with ethdium bromide.

The polymorphism was genotyped via restriction digestion with *Nla*III endonuclease according to the manufacturer's protocol. The digested products (allele A site present) were electrophoresed on a 6% agarose gel (made using NuSieve®3:1 agarose, BMA) and visualised with ethidium bromide staining.

8.3 Results

8.3.1 Allele frequencies

Allele frequencies were similar to previously reported values, with the *COMT158*^{met} allele occurring slightly less frequently than the *COMT158*^{val} allele in the total study population. Separate chi-square analyses were conducted to ascertain whether allele frequency for the single copy (on the non-deleted chromosome 22) of *COMT* in 22q11DS individuals differs from that expected in the whole population. There was no significant difference in observed versus expected frequencies in 22q11DS either in comparison to the control population (p=0.56), or in comparison with parents of 22q11DS subjects (p=0.13). Genotypes were not available for all subjects, either because they declined to provide samples for genetic analysis, or because of low DNA yield from buccal swabs, or because of failure of PCR reactions.

Table 8.1 *COMT*^{met}*158*^{val} allele frequencies in 22q11DS and control groups

Group	Total number of alleles typed	Number of Met alleles (%)	Number of Val alleles (%)
22q11DS	26	14 (54)	12 (46)
Controls	28	17 (61)	11 (39)
Parents of 22q11DS subjects	59	21 (36)	38 (64)
Total	109	50 (46)	59 (54)

8.3.2 22q11DS-*COMT*^{met}*158*^{val} subgroup characteristics

Groups were well matched for age and gender. There was a trend towards lower estimated IQ for the 22q11DS^{met} group, although post-hoc comparisons (Bonferroni corrected) indicated no significant difference between controls and 22q11DS^{met} (p=0.07), nor between 22q11DS^{met} and 22q11DS^{val} groups (p=0.5).

Table 8.2 22q11DS-*COMT*^{met}*158*^{val} subgroups - gender, age and IQ

	Group			*Chi-square / ANOVA F*
	Controls	**22q11DS^{met}**	**22q11DS^{val}**	
n	24	13	11	
Gender (male : female)	16 : 8	8 : 5	6 : 5	*0.63*
Age	16.1 (2.9)	16.6 (2.3)	17.2 (2.3)	*0.71*
IQ	72.3 (14.8)	61.8 (13.5)	70.6 (10.3)	*2.8*

8.3.3 Association between *COMT*^{met}*158*^{val} genotype and psychiatric diagnoses

There was no evidence that *COMT* genotype in the context of 22q11DS increases the likelihood of obtaining at least one psychiatric diagnosis, nor the likelihood of obtaining any one particular diagnosis, nor influences the number of diagnoses obtained (Table 8.3). Dimensional symptom counts also did not differ between 22q11DS subjects divided on the basis of genotype (Table 8.4).

Table 8.3 22q11DS-*COMT*^{met}*158*^{val} subgroups - rates of psychiatric diagnoses

		Group			*Chi-square / Kendall's tau-b* [a]
		Controls	**22q11DS^{met}**	**22q11DS^{val}**	
At least one diagnosis [b]		5 / 17	9 / 3	9 / 2	*0.16*
ADD [b]		4 / 17	6 / 5	5 / 5	*0.04*
Any mood disorder [b]		0 / 24	5 / 7	4 / 7	*0.07*
Any anxiety disorder [b]		2 / 22	4 / 8	5 / 6	*0.35*
OCD [b]		0 / 24	1 / 11	1 / 10	*0.004*
Number of diagnoses	0	17	3	2	*0.04*
	1	4	3	6	
	2	1	6	1	
	3	0	0	2	

[a] analyses contrasting 22q11DS subgroups only [b] n present / n absent

Table 8.4 22q11DS-*COMT*^{met}*158*^{val} subgroups - psychiatric symptom scores

Mean (s.d.)	Group			ANOVA F[a]
	Controls	22q11DS^{met}	22q11DS^{val}	
Total CAPA symptom score	4 (3.8)	11.6 (5.8)	11.1 (6.1)	0.03
CAPA Mood score (Z)	-0.6 (0.2)	0.6 (1.0)	0.5 (1.3)	0.32
CAPA Anxiety (Z)	-0.3 (0.6)	0.2 (1.4)	0.4 (1.1)	0.10
CAPA Attention (Z)	-0.5 (0.9)	0.6 (1.0)	0.3 (0.9)	0.08
Total Premorbid Adjustment score	7.4 (2.2)	10.6 (2.8)	12.8 (4.2)	2.3

[a] analyses contrasting 22q11DS subgroups only

8.3.4 Associations between *COMT*^{met}*158*^{val} genotype, schizotypy and specific symptoms

Mean schizotypy scores were 2.4 (s.d. 1.7) for 22q11DS^{val} subjects and marginally increased at 3.1 (s.d. 2.0) for 22q11DS^{met} subjects. This was a non-significant within-group difference (Mann-Whitney Z=-0.7). In addition, there were no differences between genotype subgroups in the likelihood of experiencing specific symptoms that were prevalent in the 22q11DS group and potentially associated with neuropsychiatric vulnerability. 4 out of 11 22q11DS^{val} subjects and 7 out of 13 22q11DS^{met} subjects reported psychosis-like phenomena (chi-square value 0.7). 5 out of 11 22q11DS^{val} subjects and 3 out of 13 22q11DS^{met} subjects reported frequent episodes of anger or aggression (chi-square value 1.3). Hence in this sample of adolescents without severe psychiatric disturbance (i.e. not medicated or receiving intensive mental health support), *COMT* genotype did not modify psychological function.

8.3.5 Association between *COMT*^{met}*158*^{val} genotype and cognitive performance

Working memory index scores, which differed between 22q11DS and controls and were weakly associated with schizotypal features (but only after covarying for IQ, which is strongly associated with working memory), showed no evidence of differentiating between 22q11DS^{met} (mean = -0.2, s.d. = 0.9) and 22q11DS^{val} (mean=-0.1, s.d.=0.6) subgroups. One-way ANOVA comparing both genotype

subgroups to the control group revealed only a trend towards between-group difference ($F[2,45]=1.9$, $p=0.16$) therefore post-hoc comparisons were not justified. Univariate analysis co-varying for IQ reduced this effect still further ($F[2,44]=0.95$, $p=0.40$). The same series of analyses was conducted for Dot Test error score, the single working memory task for which there was a significant group difference, again revealing no between-group effect ($F[2,42]=1.9$, $p=0.16$), reduced further on covariance with IQ. Therefore working memory, as assessed in this investigation and in this population, displayed no evidence of modulation by *COMT* genotype in 22q11DS.

8.3.6 Association between $COMT^{met}158^{val}$ genotype and auditory ERPs

Inspection of grand average difference waveforms (Figure 8.1) elicited by deviants in both tone and speech ERP experiments suggested that for duration MMN, $22q11DS^{met}$ subjects elicited no prominent MMN at frontocentral electrodes, whereas waveforms for $22q11DS^{val}$ subjects were similar to or intermediate with controls. Similarly, average frontocentral voicing MMN was much more markedly reduced in $22q11DS^{met}$ subjects than in $22q11DS^{val}$ subjects relative to controls. No similar effect was seen for duration MMN recorded at temporal electrodes. Table 8.5 presents the results of repeated measures ANOVAs for the ERP measures that differed between 22q11DS cases and controls: N1 (peak amplitude of tone and speech N1a at electrodes A1 and A2), frontal tone MMN (peak amplitude for duration, frequency and duration + frequency at electrode Fz) and frontocentral voicing MMN (peak amplitude for ta and ga voicing deviants at electrode Cz). For each of these measures, there was a significant main effect of genotype, with no interactions between genotype and stimulus. Post-hoc comparisons indicated significant differences only between controls and $22q11DS^{met}$ subjects. ANOVA for P3a amplitude elicited by the duration + frequency deviant at Cz was also conducted because of the existing evidence that this ERP component is affected by *COMT* genotype in schizophrenia and in the general population, and the trend towards a significant between-group difference. There was no evidence for an effect of genotype on this measure.

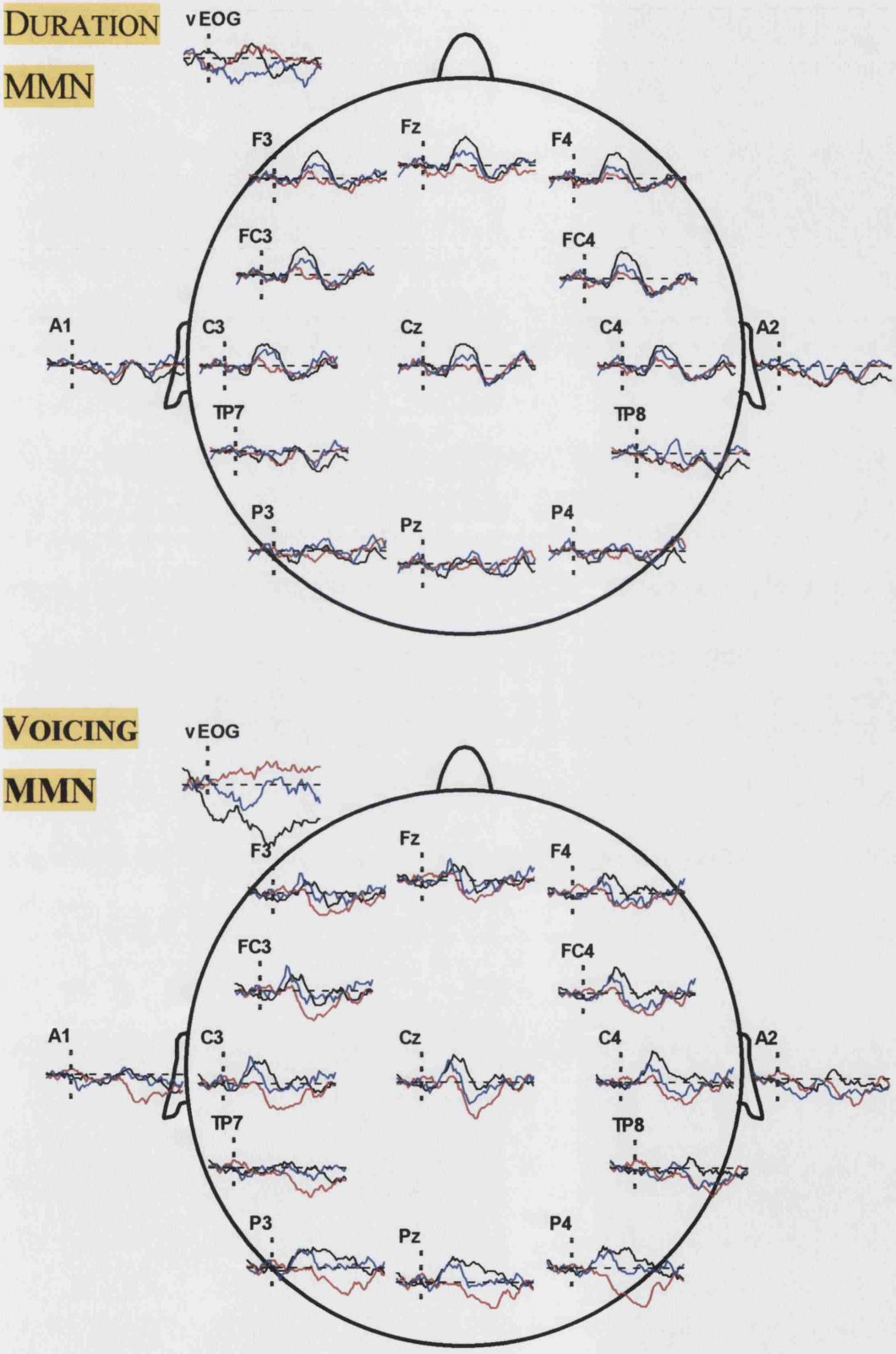
The apparent effect of *COMT* genotype as a modifying influence on case-control ERP differences could have been distorted by small group sizes (particularly for the 22q11DS^{val} subgroup). Therefore additional analyses were carried out to assess the presence and consistency of a between-subgroups effect of *COMT* genotype on N1 and MMN in 22q11DS subjects, without additional comparison with the control group. Peak amplitudes of N1a and N1c (tone and da standard stimuli) at electrodes A1 and A2 did not display any modifying effect of *COMT* amplitude within the 22q11DS group ($F[1,10]=0.08$, $p=0.8$). Peak amplitudes for duration MMN (at electrodes Fz, F3, F4, Fc3 and Fc4) and voicing MMN (at electrodes Cz, C3, C4, Fc3 and Fc4) were assessed for within-group effects, because these ERP components showed the strongest test-retest reliability in normal adults, and also displayed an association with schizotypy within the 22q11DS sample. Tone N1a amplitude (electrode A2), was entered as a covariate, since this was found to be a confounding factor in the initial case-control comparison, and also showed a weak effect of *COMT* genotype in the whole-sample analysis. A difference approaching significance was detected in the magnitude of responses between 22q11DS^{val} and 22q11DS^{met} subjects ($F[1,12]=4.1$, $p=0.065$). There was no interaction between genotype and stimulus ($F[1,12]=0.05$, $p=0.8$), nor between genotype and electrode ($F[3,35]=1.2$, $p=0.3$).

An additional difference between genotypes was identified on visual examination of waveforms in the speech condition. The 22q11DS^{met} subgroup displayed a marked positive deflection in both mean speech ERPs (voicing and place of articulation), with a peak latency of approximately 250ms post stimulus-onset. Repeated measures ANOVA, including data for both 22q11DS subjects and controls, comparing the amplitude of positive peaks in the time-window 200-400ms, indicated a highly significant effect of *COMT* genotype ($F[2,27]=8.7$, $p=0.001$). Post-hoc comparisons confirmed that this difference was driven predominantly by the 22q11DS^{met} subgroup, who differed significantly from controls ($p=0.001$) and almost differed significantly from 22q11DS^{val} subjects ($p=0.07$).

Table 8.5 22q11DS-*COMT*^{met}*158*^{val} subgroups - auditory ERPs

ERP component	Stimuli	Electrode(s)	ANOVA				
			df	F	p	post-hoc comparison	p
N1a	tone, da standards	A1, A2	2,27	5.6	0.01	controls > 22q11DS^{met}	0.01
						controls = 22q11DS^{val}	0.11
MMN	dur, freq, dur + freq deviants	Fz	2,26	3.5	0.04	controls > 22q11DS^{met}	0.08
						controls = 22q11DS^{val}	0.18
MMN	ta, ga (voicing) deviants	Cz	2,28	4.0	0.03	controls > 22q11DS^{met}	0.03
						controls = 22q11DS^{val}	0.63
P3a	dur + freq deviant	Cz	2,27	1.6	0.23	not justified	

Figure 8.1 Effect of *COMT*^{met}*158*^{val} genotype on auditory ERPs in 22q11DS

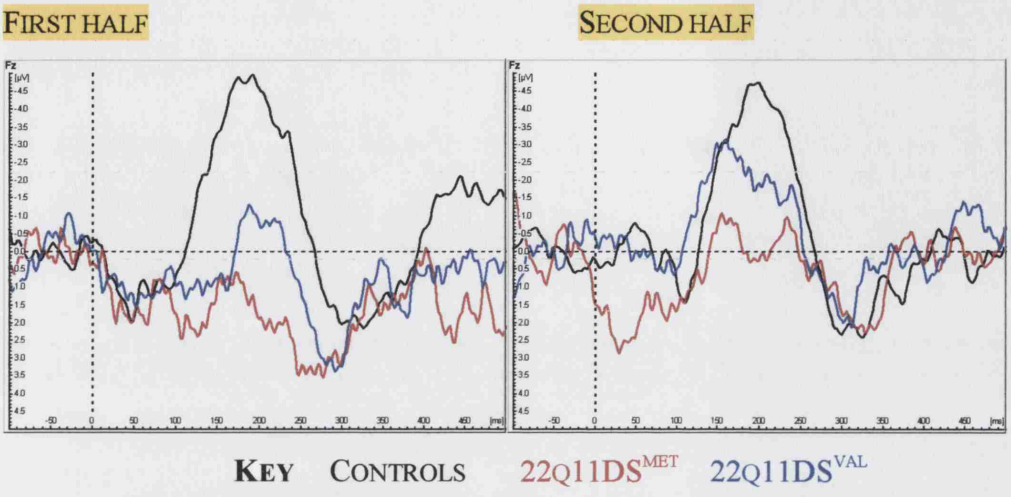


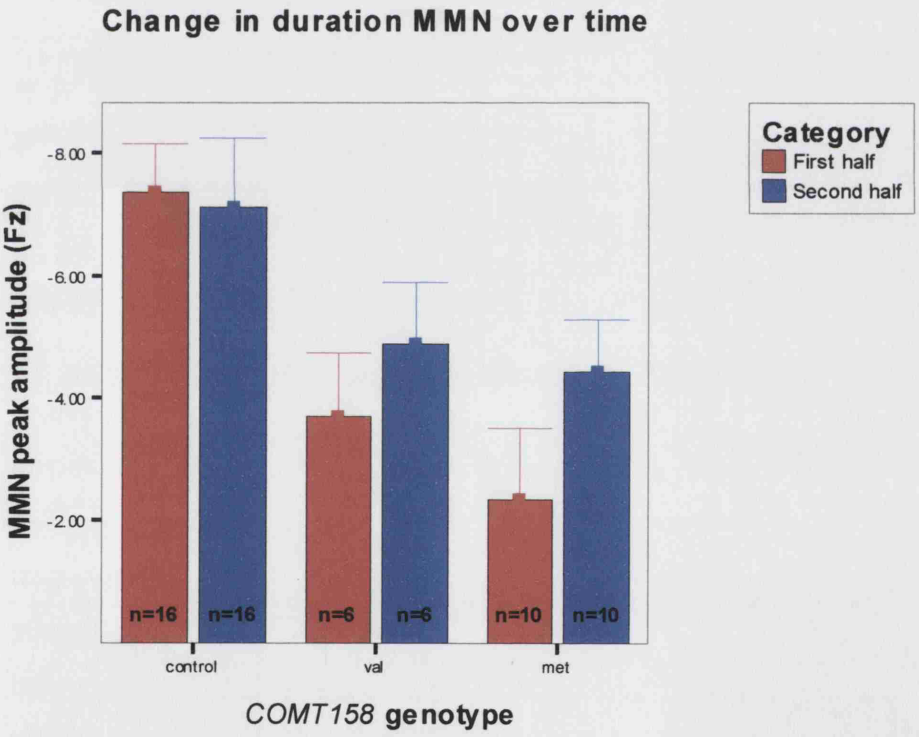
8.3.6.1 Association between *COMT*^{val}*158*^{met} genotype and change in auditory ERPs across the testing block

In the initial case-control study, differences between 22q11DS subjects and controls in the magnitude of duration MMN were marked during the first half of the testing block, with responses in the 22q11DS increasing to close to control-level in the second half. Therefore one hypothesis to explain the effect of *COMT* genotype on MMN amplitude would be that individuals expressing only one copy of the low-activity COMT enzyme initially do not generate frontal MMN, but improve considerably over the course of the testing block. Alternatively, both 22q11DS subgroups might initially display diminished frontal MMN, with only 22q11DS^{val} subjects improving over time. Observation of waveforms, however, suggested a third possibility – both 22q11DS subgroups displayed reduced frontal MMN during the first half of the testing block, and both subgroups increased the magnitude of the deviant-related response in the second half of the block. During both phases, 22q11DS^{met} subjects displayed smaller frontal duration MMN than 22q11DS^{val} subjects and controls.

A second repeated measures ANOVA was conducted to test this observation statistically, comparing peak MMN amplitudes for the duration deviant during the first and second half of the testing session (at electrode Fz) within the 22q11DS group only. This confirmed a significant change across the testing session in 22q11DS subjects, with larger responses in the second half of the testing session (main effect of testing phase $F[1,14]=5.0$, $p=0.04$), with no interaction between *COMT* genotype and phase ($F[1,14]=0.4$, $p=0.6$). However this analysis did not reveal a main effect of genotype ($F[1,14]=0.4$, $p=0.5$), indicating that with small group numbers, and small numbers of trials available for each individual subject in the split-half analysis, signal-to-noise ratio may be too low to detect between-subgroup differences. Entering data from control subjects into a final repeated measures analysis of duration MMN at Fz in the two halves confirmed that whilst 22q11DS^{met} subjects differ from controls (Bonferonni corrected $p=0.006$), 22q11DS^{val} subjects do not ($p=0.11$) (Figure 8.2).

Figure 8.2 Change in duration MMN over time, effect of *COMT* genotype





8.4 Discussion

8.4.1 Summary

- In the sample of adolescents investigated here, none of whom were suffering from severe psychiatric disturbance at the time of assessment, but many of whom were displaying mild to moderate features of schizotypal personality and prodromal-like symptoms, no modifying impact of *COMT*^{val}*158*^{met} genotype on psychiatric phenotype was detectable. Approximately equal numbers of subjects for each of the two alleles reported psychosis-like experiences, suffered from mood disturbance or anxiety, and displayed poor psychosocial adjustment.
- Although the number of subjects in whom it was possible to investigate genotype-endophenotype associations was very small, a consistent pattern emerged. For both duration and voicing MMN, the parameters previously found to differentiate cases from controls and to be associated with degree of schizotypal personality, the most marked impairments were seen in 22q11DS^{met} subjects, whilst 22q11DS^{val} subjects displayed ERPs that were at intermediate levels with respect to controls.
- The effect of *COMT* genotype on frontocentral duration MMN amplitude within the 22q11DS group was seen during both the first and second halves of the testing block. In comparison to controls, both subgroups increased MMN amplitude over the course of testing.
- For speech contrasts, 22q11DS^{met} subjects elicited a marked positive peak, maximal at frontocentral electrodes with a latency of approximately 250ms post stimulus-onset. This may be an ultra-fast P3a-like indicator of transition of information through attentional networks, or may be aberrant activity of unknown origin. A similar enhancement of P3a-like activity was not seen for pure tone stimuli.
- No effects of *COMT* genotype within the 22q11DS group were detected for either N1 (impaired in both subgroups) or P3a (significantly impaired in neither subgroup).

8.4.2 Limitations

- The small sample of 22q11DS individuals investigated here renders these results preliminary, especially with respect to genetic-neurophysiological relationships.
- Testing of a much larger sample of general population controls is required to assess any potential relationship between *COMT* genotype and the key ERP variables examined here. For reference, it is worth noting that Weinberger and colleagues required a sample of several hundred individuals to demonstrate an extremely marginal positive effect of the 22q11DS^{met} allele on WCST performance (Egan et al 2001).
- Comparison with additional populations, in particular idiopathic adolescent psychosis, would be especially informative regarding the consistency of any modifying impact of genotype on cognitive function in high-risk individuals.
- The method used to obtain DNA samples and assign genotypes did not generate 100% yield. Two subjects from the 22q11DS sample did not return their sample packs. Of those who did, genotypes could not be assigned in 5 individuals, because of poor quality DNA extraction or failure of PCR, despite repeated attempts and the use of two primer sets.
- The cognitive tasks administered in this investigation may not have been sensitive to genotype effects. In previous studies demonstrating that *COMT* contributes to individual differences in prefrontal function, either WCST or delayed-response working memory tasks have been used. A particular advantage of these latter tests (both the N-back numbers task, and spatial delayed response tasks) is that working memory load can be controlled parametrically, so that the impact of genotype can be assessed at varying levels of cognitive effort and success. Design of such a task for use in young and learning disabled populations, in which performance profiles are independent of IQ even if absolute levels of performance vary, would be challenging but worthwhile for future studies.
- Although the ERP-genotype relationships were consistent across stimulus-types and electrodes, the between-genotypes effect within the 22q11DS group could have been driven by a small number of subjects who differed from the remainder on some critical factor other than *COMT* genotype.
- Groups studied here were much too small to assess potential interactions between *COMT* genotype and other factors, for example gender, or other polymorphisms within *COMT* or other genes.

8.4.3 Implications

COMT is a good candidate gene within the 22q11 region for disruption of neurobiological function and a consequential increase in psychiatric risk in 22q11DS. However, obtaining direct evidence for this hypothesis is difficult. The *COMT*^{val}*158*^{met} polymorphism, known to impact on cognitive function and psychiatric risk in the general population, may mediate within-group differences in outcomes by altering the individual's ability to compensate for haploinsufficiency. In this study we have demonstrated, albeit in a small population, that *COMT* genotype modifies the expression of neurocognitive endophenotypes relevant to schizophrenia-risk, given the presence of a microdeletion at 22q11 and thus *COMT* haploinsufficiency. The 22q11DS *COMT158*^{met} carriers, who are presumed to have a very low capacity for dopamine clearance in the prefrontal cortex, showed markedly abnormal frontal MMN, which was not the case for *COMT158*^{val} carriers. Both subgroups displayed reduced N1 amplitudes, and neither subgroup displayed significant P3a amplitude differences relative to controls. This supports the general principle that neurocognitive markers may have more power in identifying genetic risk factors than diagnoses or symptoms.

Although these results enhance the existing data suggesting that *COMT* is a gene of influence for neural and cognitive function, potentially relevant to psychiatric disorders, they also contradict previous reports in certain respects. The data collected by the Weinberger group has implicated the *COMT158*^{val} allele in inefficient prefrontal cortex activation during working memory tasks in the general population, schizophrenia patients and their relatives. It has been demonstrated here that, in 22q11DS at least, the *COMT158*^{met} allele can also be disadvantageous. This suggests either than the polymorphism behaves differently as a modifying influence in the context of different genetic backgrounds, or that each allele is advantageous under some information processing demands whilst being deleterious in others. This latter view is supported by the experiment presented by Mattay et al regarding genotype-influenced neural and cognitive consequences of amphetamine administration. An additional experiment contrasting the MMN paradigm with a working memory task and fMRI analysis similar to that employed by Weinberger et

al would test the hypothesis that, under different information processing demands, 22q11DS individuals with either *COMT* allele may be more impaired.

In a similar fashion to the data presented here, Gallinat et al (2003) demonstrated that the *COMT158*^{met} allele is associated with abnormal auditory oddball ERPs, an effect seen most clearly in individuals with schizophrenia. However, this previous report found an effect for the P3 component, which has not been detected in the current experiment. One explanation for this contradiction is that the current study is underpowered to detect differences in P3a, because of the high degree of individual variability and low test-retest reliability of this ERP. A second possibility could be that abnormalities in the P3 component could emerge later in development in the context of declining psychiatric and cognitive function, and that this state-related emergence of ERP abnormalities in the context of a vulnerable neurodevelopmental state could be influenced by *COMT*. A last possibility is that the contrasting pattern of *COMT* genotype influences (on MMN in 22q11DS, and P3 in schizophrenia) could reflect actual population differences in the nature of neurobiological abnormality and the actions of this risk-related gene. Gallinat did not report MMN data, therefore a direct comparison cannot be made regarding the effect of *COMT* polymorphisms on the earlier ERP component in 22q11DS, schizophrenia and normal adults.

The finding that both genotype-defined subgroups of 22q11DS subjects displayed a similar pattern of dynamic change in MMN across the testing block points towards quantitative rather than qualitative allele-mediated differences. The group-wide enhancement over time (in duration MMN amplitude at frontal electrodes across the testing block) suggests that synaptic dopamine clearance provides a stable, modulating influence on the emergence of the deviant-related auditory ERP. It has been shown that, in 22q11DS, this process is not absolutely disabled, but that its efficiency is reduced such that it takes longer for the change-related ERP emerge over the course of the testing session. This capacity appears to be most reduced for 22q11DS subjects carrying the low-activity *COMT158*^{met} allele on their non-deleted chromosome 22. The *COMT* subgroup-parallel increases across the testing block indicate that establishment of reliable frontal MMN over a time-period of minutes is driven primarily by an additional, COMT-independent factor that does not differ between subgroups.

The strongest candidate for this "driving force" is glutamatergic input, given the existing evidence for the dependence of MMN on NMDA receptor activation (Javitt, Steinschneider, Schroeder, & Arezzo, 1996). According to work by Goldman-Rakic and colleagues (2000), dopaminergic modulation of NMDA-dependent glutamatergic synapses is responsible for the inverted U-shaped function of optimal working memory function in the prefrontal cortex. This modulation occurs via D_1 receptor activation at pyramidal cell dendrites and inhibitory interneurons, changing the likelihood of NMDA-receptor activation via voltage-dependent and intracellular signalling mechanisms. It is proposed that in 22q11DS, inadequate dopamine clearance in local networks of this type (which may be in the prefrontal cortex or elsewhere) leads to supra-optimal stimulation of D_1 receptors leading to feed-forward inhibition of pyramidal cell firing. These dysregulated modulatory influences may impair the wider network-based adaptive process by which responses to standard stimuli are suppressed and responses to deviant stimuli are facilitated, however the network-based mechanism by which this occurs is unknown. A direct test for *COMT*-genotype influenced interactions between dopaminergic and glutamateric systems during MMN generation would be to challenge the auditory ERP system with ketamine. This NMDA antagonist has been shown to reduce MMN initiation following direct cortical application to monkeys (Javitt et al 1995), and systemic administration in humans (Umbricht et al., 2000). From the experiment presented here it would be predicted that individuals with high COMT activity (22q11DS subjects carrying the $COMT158^{val}$ allele or $COMT158^{val/val}$ in the general population), a higher ketamine dose would be required to suppress MMN elicitation than in individuals with low COMT enzyme activity.

COMT genotype in 22q11DS was observed to modify the severity of neurocognitive endophenotype, but not psychiatric manifestations. Both genotype-defined subgroups showed evidence of psychological and cognitive disruption. This means that *COMT* hemizygosity may not be sufficient to account for increased risk of psychosis in this population. Two models are compatible with these preliminary results. *COMT* hemizygosity may be sufficient for psychosis-vulnerability, and genotype-influenced quantitative differences reflect additional modification of risk. Alternatively, haploinsufficiency of a second gene in the typically deleted region

may cause the primary disruption, *Tbx1* being a reasonable candidate gene for neurodevelopmental effects, with *COMT* acting as an additional modifying influence.

COMT may be a critical gene influencing individual differences in cortical function, via regulation of catecholamine flux under stable and dynamic conditions. However, the dissociation between effects of genotype on neural and behavioural features in 22q11DS indicates that this gene is likely to interact with other factors in a complex fashion to mediate vulnerability to psychosis. Whilst genotype-endophenotype associations should be relatively stable, the overt expression of the vulnerable state may fluctuate over time or may not emerge until a later stage of development. The lack of significant association between genotype and psychopathological features in young people with 22q11DS, in contrast to effects of genotype on neurocognitive function, suggests that as one moves through levels of analysis, from genetic to neural, and from neural to cognitive and experiential, interactions between systems and impact of environmental circumstance increase. The most challenging task for future investigation will be to integrate genetic and physiological models with social and environmental contributors, to relate mechanisms at each level of analysis to normal and abnormal subjective experience.

9 General Discussion

In this final section, a brief review of the investigation will be presented, referring to the hypotheses and aims established in Chapter 1, and bearing in mind general limitations. Some ideas for future investigations and clinical implications will then be presented, followed by concluding comments regarding the approach adopted in this study and its potential application to other areas of developmental neuropsychiatry.

9.1 Have schizophrenia-like features been observed in adolescents and young adults with 22q11DS?

At the outset of this investigation, there was very little evidence for schizophrenia-like or psychosis-like disruption in 22q11DS individuals who did not have a full diagnosis of a psychotic illness. For each experiment in which 22q11DS adolescents and young adults were compared to age- and IQ-matched control subjects, some degree of similarity was observed between 22q11DS-control differences and schizophrenia-like features as predicted from literature review. This similarity has been observed as both overt psychopathological and underlying neurocognitive disruptions. Thus the evidence in support of overlapping developmental features in the syndrome and the idiopathic illness is considerable. This contributes some additional empirical evidence to the general neurodevelopmental hypothesis for schizophrenia, if one considers 22q11DS to be equivalent to any other high-risk group.

It is important to note the potential for myopia contributing to this interpretation as a consequence of methods and measures selected and the presumption of similarity between groups, established on the basis of previous investigations. There is a need to confirm directly the overlap between these features in 22q11DS and in adolescent-onset psychoses and other high-risk individuals, and to confirm which features in 22q11DS are distinct from those of other developmental disorders. The extent of observed potential overlap and limitations to this interpretation for each experiment are reviewed below.

9.1.1 Psychopathology

In Chapter 3, diverse psychiatric symptoms and a broad spectrum of associated impairments in familial, social and occupational function were reported for 22q11DS individuals, which were not seen in control subjects with equivalent levels of learning disability. Symptom profiles were extracted from CAPA assessments according to the criteria for ICD-10 Schizotypal Disorder, and according to Premorbid Adjustment Scales commonly used in schizophrenia research. These methods were selected both because they were descriptively coherent with the spectrum of features reported for 22q11DS, and because of pre-existing evidence for a relationship between the syndrome and schizophrenia. Therefore the use of these scales may artificially prejudice the characterisation of psychopathology in 22q11DS within the established framework of schizophrenia. Notwithstanding this caveat regarding interpretations, this method allowed for the quantification of diverse features that were found to be very prevalent in the 22q11DS population, including psychosis-like phenomena, affective instability and social dysfunction.

Whether the characterisation of psychopathology presented here is really schizophrenia-like depends on the concepts and definitions applied to this term. Key issues that remain disputed are the distinction between schizophrenia and affective psychoses, especially during adolescence and in the emergent phase of an illness, the definition of schizophrenia-spectrum disorder as a developmental state continuous across time within the individual, and the concept of schizophrenia-spectrum features as continuous with psychological diversity in the general population.

If it is taken as a true statement that 22q11DS is a population at high-risk for schizophrenia, then the data presented here support an inclusive attitude to affective and psychotic symptoms within the schizophrenia-spectrum, and support developmental continuity between adolescent and adult disruption. On the other hand, observation of this cohort as they progress into adulthood, and further characterisation of the adult psychopathology of 22q11DS, may reveal an ongoing tendency towards diverse psychiatric disorders, meeting criteria for schizophrenia in only a minority of cases, or representing a unique and specific psychopathological phenotype, or remaining ambiguous and changing over time within individuals. If

any of these scenarios is true then there may be developmental continuity within 22q11DS individuals, but less reason to argue for continuity between 22q11DS and idiopathic schizophrenia. Longitudinal assessments are required to establish whether continuity is in fact superseded by psychopathological deterioration or the evolution of new and developmentally unpredictable disruptions in some 22q11DS individuals.

With regard to continuity between the atypical psychological states observed in 22q11DS subjects and "normal" function, it is significant that no bimodal distributions were observed in schizotypy, symptom-score or premorbid adjustment data, indicating no distinctly "abnormal" subgroup despite very considerable variation in type and severity of symptoms within the sample. Additionally, many of the features considered to be "symptoms" were ambiguous, in their relationship to normal child and adolescent behaviour (when, in terms of degrees of severity and developmental age, does a temper tantrum or a parental argument become impulsive aggression and depressive anger?) and to unusual but acceptable personality features (when does fantasy or suspicious thinking become delusion? when does a lonely and socially unskilled adolescent become a recluse?). However, these features were not seen in the control population, indicating that they are found relatively infrequently in the general population. These features were also, in many cases, distressing and disruptive to individual and family life, therefore to dismiss them as aspects of normal diversity is to undermine their personal significance.

9.1.2 Neurocognitive impairments

Observing similarity between 22q11DS and schizophrenia with respect to neurocognitive traits (potential endophenotypes) is, on one hand, an easier proposition, because of the use of objective measures and controlled testing procedures that are less likely to be influenced by social and environmental factors. However it is also possible that, in moving from subjective to objective phenomena, there is increasing divergence between 22q11DS and idiopathic psychoses. Schizophrenia, defined as a subjective mental state or set of similar mental states, does not presume parallel similarity in underlying pathophysiology. Lack of clarity in the literature as to what constitutes a schizophrenia-like neurocognitive state, and heterogeneity in disruption amongst patient populations, limits the validity of

interpretations based solely on literature, and increases the need for direct experimental comparisons between 22q11DS and psychiatric patient populations.

Despite these potential pitfalls, the cognitive and neurophysiological experiments conducted here provided considerable additional support for similar developmental disruption in 22q11DS and schizophrenia. Cognitive testing revealed a tendency towards impoverished working memory function in 22q11DS, associated with poor expressive language function. Neurophysiological testing provided strong evidence for a schizophrenia-similar pattern of dysfunction, with selective diminution of mismatch negativity at frontocentral electrodes, elicited by deviants in both physical and phonetic features in a repetitive sound stream. Although some disruptions to auditory and speech ERPs were seen in children with Specific Language Impairment, these differed from those observed in 22q11DS and those reported in the schizophrenia literature, and showed a distinct pattern of cognitive-ERP relationship in this additional comparison population. Taken collectively, these results suggest that, in 22q11DS, specific neurobiological processes relating to information processing are disrupted, either in a regionally specific manner (most likely implicating the prefrontal cortex) or in a regionally non-specific manner involving a common repertoire of molecular mechanisms.

However, there are considerable limitations in interpreting these observations in 22q11DS as confirming the presence of schizophrenia-like disruption. No individual working memory task was performed at markedly impaired levels in the majority of 22q11DS subjects. Significant working memory dysfunction was only seen when results from several tests were combined into an average sample-standardised score. There was still very considerable overlap in scores demonstrated by 22q11DS and control subjects for this measure, and no independence from general cognitive ability in either group. Working memory and language disorders cannot be considered specifically schizophrenia-like as they are seen in other developmental disorders which are not associated with especially high rates of psychosis in adulthood. Therefore either working memory is not a useful cognitive construct for investigation as a psychiatric endophenotype, or much more specific measures of the type and severity of working memory dysfunction are required.

Although frontal MMN abnormalities provided strong evidence for similarity between 22q11DS and schizophrenia, several issues require clarification. Firstly, N1 abnormalities were also seen in 22q11DS, and P300 abnormalities were not, a pattern not generally associated with schizophrenia or psychosis-risk. Secondly, there is limited evidence for speech processing abnormalities, of the type seen in 22q11DS, as a neural component of schizophrenia. Thirdly, the pattern of dynamic change in MMN elicitation, indicating inefficient rather than entirely impaired processing, has not been previously reported in a psychosis-prone population. ERP abnormalities as observed in 22q11DS may be shared with at least some individuals with idiopathic psychoses, or may be a profile of neurobiological disruption that is specific to the syndrome, but comparison data for appropriate populations is not at present available. Although MMN abnormalities at the diagnostic group-level are seen in schizophrenia and not in bipolar disorder or other affective illnesses, the profile delineated for 22q11DS may be associated with a symptom dimension or cognitive disruption that cuts across these categories. To address these issues, direct comparison with a variety of psychiatric patient populations and other at-risk individuals is required, using the same testing protocols and multiple methodologies applied to the 22q11DS population.

9.2 *What is the relationship between neurocognitive impairment and psychiatric disruption in 22q11DS?*

Both the psychopathological and neurocognitive experiments identified 22q11DS-associated impairments that appeared similar in some respects to literature-based expectations of a high-risk population for psychosis. There was considerable variation within the group in expression of these features by 22q11DS individuals, from few overt symptoms and no specific neurocognitive impairments, to many intrusive symptoms and marked neurocognitive differences from the control group. Variation within the 22q11DS group on each dimension investigated was not related to degree of general learning disability (except in the case of working memory). Neither were there distinct subgroups of 22q11DS subjects displaying markedly worse psychiatric or neurocognitive profiles. Hence it seemed plausible that a continuum of psychosis risk-related developmental disruption exists for this population, with variation within the group being quantitative and not qualitative.

Only a weak relationship, confounded by IQ-dependence, was found between working memory function and psychiatric symptoms, and no relationship was detected between language dysfunction and symptoms (other than degree of social impairment). This suggests that either the measures used to index these cognitive phenomena were not accurate enough to quantify the degree of disruption to the same extent as was possible for ERP measures, or that these domains of abnormal function are unrelated to psychosis-risk in 22q11DS. The additional lack of convergence between ERP and cognitive measures in this population (in contrast to that achieved in SLI), indicates that an independent set of vulnerable developmental mechanisms may be responsible for language and communication impairments in the syndrome. However, there may be multiple confounding factors mediating within-group variation in language outcome, including success of surgical and therapeutic interventions for articulation disorders, degree of learning disability, educational provision and social environment. Given the likely powerful and interactive impact of these factors, it seems improbable that behavioural measures of language performance would be reliable indicators of vulnerable neurobiology.

Within-sample correlations for the 22q11DS group suggested that the degree of neural abnormality, as indexed by diminished amplitude of frontocentral MMN elicited by duration or voicing deviants, is associated with the number of schizotypal features demonstrated. This association does not, of course, infer causality of one factor for the other. Convergence does, however, indicate that both types of abnormality are reflections of disruption to a common physiological substrate. The quantitative variation within the 22q11DS group along both types (neural and psychological) of pathological dimension encourages speculation about the nature of the physiological substrate, and the factors that cause disruption and mediate within-group variation in the integrity of this substrate. Although convergence between psychiatric and neurocognitive abnormalities within the 22q11DS implicates common mechanisms underlying vulnerability observed at these different levels of analysis, no strong claims that these observations reflect relative psychosis-risk within the 22q11DS population without longitudinal assessments of psychiatric outcomes.

9.3 *Using the endophenotypes approach to identify a genetic mediator of neuropsychiatric risk in 22q11DS*

Frontocentral duration MMN corresponds to our definition of an endophenotype – a genetically-influenced continuous trait associated with expression of psychiatric symptoms – both in idiopathic schizophrenia and, as shown here, in 22q11DS. Therefore it has validity as a marker for the identification of causal factors mediating within-group differences. Voicing MMN, not previously investigated in schizophrenia and potentially a unique feature of 22q11DS because of specific disruption during development, also corresponds to this definition. The voicing MMN deficit is only partially independent of duration MMN, as indicated by correlations between these two measures and between each measure and schizotypal features, but with each component displaying different topography across the scalp. This partial convergence suggests dependence on a common molecular mechanism of disruption, employed in distinct neural circuits, with potentially distinct impact on cognitive function and subjective experience.

As demonstrated and discussed in Chapter 8, quantitative variation in frontocentral (duration and voicing) MMN abnormality between 22q11DS subjects was found to be mediated by *COMT*^{val}*158*^{met} genotype on the lone, non-deleted chromosome 22. Even though the population under investigation was very small, subjects carrying the *COMT158*^{met} allele, associated with low-enzyme activity and reduced catecholamine clearance, particularly in the prefrontal cortex, differed significantly from the remainder of the 22q11DS group. Since both genotype-defined subgroups demonstrated similar improvements in MMN over the course of the tones testing block, it is proposed that COMT enzyme deficiency, as a consequence of haploinsufficiency, impairs the adaptive process by which MMN emerges. Reduced clearance of dopamine from symmetrical glutamatergic-dopaminergic synapses, found at various critical locations in the cerebral cortex, may render inefficient the process of synaptic potentiation and network-based tuning by which responses to standard, repeated sounds are inhibited, and responses to deviant but non-novel sounds are facilitated. The impact of the *COMT*^{val}*158*^{met}genotype on enzyme activity levels and on functional modulation of cortical information processing in the general population, in schizophrenia and relatives of schizophrenia patients, and now in 22q11DS, further extends our understanding of the relationship between

catecholamine regulation, neurocognitive function and psychiatric vulnerability. However this experiment does not prove that *COMT* is the critical hemizygous gene causing cognitive and psychiatric disruption in 22q11DS, as this data is also compatible with a multi-gene model, with *COMT* modifying the functional impact of another critical factor (possibly *Tbx1*).

Small sample size requires that this study be replicated independently. Larger samples will also facilitate the investigation of interacting factors, such as gender and additional genotypes either at 22q11 or elsewhere in the genome. The proposed model implicating modulation of NMDA-dependent synaptic potentiation by catecholamines, and dependence of individual differences on clearance efficiency, is inferential and speculative, and requires experimental testing via pharmacological manipulations in typical and atypical human populations and in animal models. The lack of any significant association between $COMT^{val}158^{met}$ genotype and cognitive performance or psychiatric features suggests that either this proposed genetically-mediated neurobiological vulnerability is unrelated to behavioural features in 22q11DS, or that in moving from biological to behavioural analyses, observed relationships are increasingly confounded by genetic and environmental compensating and mediating factors.

9.4 Further investigations

Investigation of younger children and older adults with 22q11DS will allow further analysis of the developmental time-window over which neurobiological vulnerability may influence the emergence of atypical cognitive and psychological function. Longitudinal assessments will establish the stability, fluctuations or deterioration in neural and psychiatric phenomena in this population. As has been alluded to earlier, a direct comparison between 22q11DS adolescents and individuals with early-onset idiopathic psychosis is necessary, in order to confirm that findings presented here are similar to those seen in the illness in the general population. Ideally, this study would be extended to other high-risk individuals, i.e. adolescent offspring or siblings of patients.

Future investigations should be extended in breadth, for example to determine whether there are additional cognitive disruptions in domains such as attention, executive function, long-term memory and social cognition, and whether other genetic factors within the 22q11 region are responsible for or mediate variation in cognitive and psychiatric features of the syndrome. The addition of MRI structural and functional imaging protocols in future investigations will introduce a new dimension to the characterisation of neurodevelopmental and functional deficits in 22q11DS that may explain vulnerability to psychosis.

Current results also need to be extended in depth, for example to address the processing parameters affecting working memory performance, to define additional experimental factors influencing the efficiency of the change-detection ERP sequence, to describe in more detail the emergence and dynamics of psychopathological symptoms, and to test additional hypotheses about regulatory elements and additional polymorphisms in the *COMT* gene. In a much larger sample it may be possible to utilise the relative dissociation between genetic effects on endophenotypic and overt features to study additional, non-genetic mediating factors that determine the likelihood of expression of disorder given the presence of a genetic and neurobiological risk-state.

9.5 Potential clinical implications

22q11DS individuals approach mental health service providers with diverse clinical difficulties. When a clearly recognisable psychiatric disorder is observed, standard treatments are applied, with variable success in improving symptoms and functional outcomes (not necessarily any different from the outcomes in other individuals presenting with the same illness). There are at least four major obstacles to clinical service provision for 22q11DS individuals who are experiencing symptoms and seeking help.

Firstly, clinicians may be of the opinion that, because 22q11DS is a recognisable aetiological factor for psychiatric disorder, some special regime may be required of which they are unaware, and they may be reluctant to treat a patient for fear of error. Evidence from this investigation suggests that there is a considerable degree of

overlap between features in 22q11DS and idiopathic psychosis, both in overt psychiatric phenomena and underlying neurocognitive disruption. This should encourage clinicians to follow their normal clinical practices, although the presence of the syndrome and the potential for currently unidentified differences and dangers should be remembered.

Secondly, we have observed in this investigation that adolescents with 22q11DS can present with symptoms cutting across several diagnostic categories, which can be severely disruptive, but may be sub-threshold for any one diagnosis or present a complicated picture of co-morbidity which may be difficult to approach given current therapeutic guidelines. So long as psychiatric diagnoses are based solely on behavioural phenomena with little regard for underlying neurobiology, this situation will not change.

Thirdly, there is a simple lack of awareness on the part of both parents and clinicians that these young people, particularly those with more severe learning disability, may be experiencing marked and disturbing emotional and psychosis-like symptoms. Young people in this study frequently reported symptoms that parents were unaware of (or were unwilling to divulge). Hence a more active investigative stance may be required of both parents and clinicians in order to effectively monitor the mental health status of 22q11DS youngsters.

Lastly, an obstacle to service provision far from specific to 22q11DS is the general scarcity of appropriate resources for diagnosis, treatment and ongoing support of young people's mental health needs in the U.K.

A potential consequence of the endophenotypes approach is that investigation of vulnerability factors underlying the emergence of psychiatric morbidity in this and other aetiologically homogeneous populations may result in the identification of novel therapeutic targets. As stated many times in this thesis, routes towards symptoms are likely to be extremely diverse, and symptomatic expression of underlying vulnerabilities may be unpredictable. Diagnosing and treating disorders on the basis of biology rather than psychological profile may lead to better outcomes with fewer negative effects. Statements regarding potential targets have been rendered purposefully vague here. It is possible to imagine that further investigation

of the mechanisms of dopaminergic dysregulation in 22q11DS, including elucidation of interactions with receptors and other neurotransmitter systems, could result in the identification of a sensitive and specific armoury of pharmacological interventions. Alternatively, if further investigation reveals that neurophysiological abnormalities alone are not predictive of psychiatric disturbance without the presence of mediating social and environmental factors, then interventions based on specific modifications of these precipitating factors may be both more effective and more acceptable. These potential applications of knowledge of 22q11DS may be valuable not only to individuals with this syndrome but to sufferers of diverse mental health problems in the general population.

This investigation has confirmed that disruptions that are distressing and functionally significant are prevalent during adolescence in 22q11DS, as in other high-risk populations. In individuals with most severe neurocognitive disruption, the likelihood of current symptomatic expression was seen to be high. This begs the question of whether interventions should be on offer for individuals displaying sub-diagnostic symptoms during this developmental period, which could both ameliorate overt difficulties and reduce the likelihood of deterioration into a more severe and chronically disabling state. A further question is whether it would ever be ethical to intervene purely on the basis of neurocognitive or even genetic risk-state, prior to the emergence of any overt symptoms.

Such questions are hotly debated with respect to early identification and pre-emptive intervention in psychosis-prone individuals in the general population (Cornblatt, Lencz, & Obuchowski, 2002). The clear danger of such an approach is that young people will be prescribed powerful psycho-active medications, for use over a large number of years. These drugs have both acute side-effects and the potential to cause chronic, negative changes in cognitive function and other aspects of mental health such as self-esteem, personality and identity. Longitudinal studies are currently being conducted at several centres around the world to assess the practicalities, benefits, safety and costs of early intervention for the general population, the results of which may well be of benefit to 22q11DS individuals and their families in opening up choices for therapeutic provision in the early phase of illness. This approach could greatly increase the likelihood of productive and happy lives for individuals with 22q11DS, and ease the considerable burden of care for families. On

the other hand it is vitally important to remember that the majority of individuals with 22q11DS do not manifest a severe psychotic illness, and that their unique personalities and novel perspectives on life, informed only in part by being carriers of the 22q11 microdeletion, may have as many positive as negative aspects.

9.6 Genes, brains and mental health – a future for integrative neuroscience?

Embarking on research that seeks to bridge the gap between a chromosomal anomaly and a complex mental disorder may be likened to the experience of a frog who, attempting to reach a lily pad, decides to jump in leaps of ever-halving distance from sequential take-off point to target. With every leap the target appears nearer, but the possibility of reaching it never improves. The frog also realises that, in taking the largest leaps first, they have missed out on observing the greatest part of the pond, and are now employed in increasing their detailed knowledge of some small and unimportant edge of the lily pad. However it may be worthwhile for the same frog, if it is endowed with a certain quality of persistence, to repeat the exercise from another point on the bank, bearing in mind what it has learnt. Still, the frog will never reach the lily pad, but it will, over time, come to recognise all of its edges and may observe changes in the pad and in the surrounding regions of pond across the seasons and over the life-cycle of the pad. In short, the frog will have become a scientist.

This investigation evolved into (undoubtedly only the start of) a fascinating journey, begun by leaping into the gulf that exists between genes and mental illness in 22q11DS. By integrating diverse techniques and literatures we may be a little closer to answering the fundamental question, stated simply: why are individuals with 22q11 Deletion Syndrome so vulnerable to developing psychotic disorders? This question is almost certainly unanswerable, at least as long as debates continue regarding the concepts involved. However, by taking a relativistic approach, and considering mental illness, not as an entirely discontinuous lesion in some "normal" state, but as a reflection of vulnerabilities and extremes of function that are intrinsic to normal human processes, one may begin to understand what circumstances make this outcome more likely. By approaching the problem at multiple analytical levels

simultaneously, we have begun to infer something of the dynamics of the atypical state, and of the nested interactions between these levels. Application of the endophenotypes approach, as defined and utilised here, results in the generation of testable hypotheses about the relationships between genes, brains and mental states in typical and atypical situations. In seeking to understand these relationships from a dynamic and integrated perspective, psychiatric genetics can move from being something akin to a modern phrenology, to a theoretically constructive and therapeutically informative science.

10 References

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